HEPATITUS B VIRUS INFECTION AMONG NATIVE INHABITANTS OF FRENCH POLYNESIA: A SEROEPIDEMIOLOGIC AND GENETIC ANALYSIS

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LIST OF ABBREVIATIONS

HBV - - - - - - - - - hepatitis B virus

HB - - - - - - - - - hepatitis B

HBsAg - - - - - - - - hepatitis B surface antigen

anti-HBs- - - - - - - antibody to hepatitis B surface antigen

HBeAg - - - - - - - - hepatitis B e-antigen

anti-HBe- - - - - - - antibody to hepatitis B e-antigen

e/anti-e- - - - - - - e-antigen and antibody system

HBcAg - - - - - - - - hepatitis B core antigen

anti-HBc- - - - - - - antibody to hepatitis B core antigen

PHA - - - - - - - - - passive hemagglutination assay

RPHA - - - - - - - - reversed passive hemagglutination assay

RIA - - - - - - - - - radioimmunoassay

I.D. - - - - - - - - immunodiffusion assay

SUMMARY

The inhabitants of four islands in French Polynesia were studied for serologic evidence of HBV infection and in order to test the hypothesis that susceptibility to becoming chronically infected with HBV is subject to genetic determinents.

Seventy percent of the inhabitants of the island of Hiva Oa and 74% of those living on Maiao were included in the study. They were estimated to be a sample representative of the total population with the possible exception that children under ten years of age were underrepresented.

Approximately 40% of the inhabitants of villages on two additional islands, Rapa and Mangareva, were also studied. It could not be estimated whether they were representative samples of their total populations.

Prevalence of HBV infection was determined by measurement of its specific serologic markers, HBsAg, anti-HBs and anti-HBc. Distribution of these markers within the study populations was expressed in terms of age and sex.

Two surveys separated in time by 19 months were conducted on the island of Maiao. This allowed for estimation of the incidence of HBV infection among susceptible persons and for a detailed pedigree analysis of the patterns of infection acquisition and distribution within families. Thus, evidence that genetic and/or environmental factors influenced host susceptibility to either the development or the maintenance of HBV infection could be considered.

HBsAg prevalence and incidence (among susceptible individuals) was 20% on Maiao while the overall infection rate was 88%. HBsAg was found in 24% of males and 15% of females while infection (HBsAg plus anti-HBs plus anti-HBc) was 93% among males and 83% among females. In both sexes, HBsAg prevalence

was greatest in the 30 - 39 year old age groups while overall infection was lowest (80% in males and 55% in females) in the 1 - 9 year old age group with a generally maintained increase thereafter, in the 90 to 100% range. This preponderance of males with HBsAg was similar to that found in many other populations throughout the world although the age of peak prevalence was somewhat greater. The finding of the lowest prevalence (and the greatest number of susceptible individuals) in the youngest subjects is at variance with some other reports where HBsAg was most prevalent in these ages. This may reflect sampling differences between this study and others.

A different situation was found to exist on the island of Hiva Oa where, although the overall infection (70%) and HBsAg prevalence(20%) rates were high, the prevalence of HBsAg among males (16%) was lower than that among females (24%) as was the infection rate (58% versus 73%). Neither HBsAg nor infection prevalence had a marked age-specific increase or decrease as was the case on Maiao, nor did those individuals acutely infected appear to be distributed by age as they were on Maiao. For these reasons it is suggested that the inhabitants of Hiva Oa, either for environmental or for genetic reasons, did not handle HBV infection in a manner similar to that observed on Maiao or in some other South Pacific populations.

Pedigree analysis of the Maiao inhabitants was consistent with the operation of an autosomal recessive inheritance of susceptibility to becoming an HBsAg-positive chronic carrier after HBV infection. However, this mechanism could not be definitively ruled in or out. Other common modes of inheritance were seen not to operate.

There was statistically significant evidence that all children, and 1 - 9

year old children in particular, of an HBsAg-positive mother were more likely to have HBsAg than those of any other parents (including fathers with HBsAg). However, infection per se was not significantly more likely to occur in progeny of a mother with HBsAg.

Limited evidence of intrafamilial spread of HBV infection was also found but could not be tested statistically due to small sample size. HLA data are pending on the Maiao subjects and it is hoped that their addition to the pedigrees will help to elucidate the contribution of genetic factors to the host response after HBV infection.

FOREWARD

In 1975, en route from Australia to the United States, I visited the island of Tahiti and while there, learned of the Institut de Recherches Médicales Louis Malardé (IRMLM), a Pasteur Institute affiliate located in Papeete and run by the French Army. The IRMLM conducts research in clinical immunology, biochemistry and marine biology, with a special emphasis on the endemic diseases which impose great burdens on the local population.

In 1977 I contacted the IRMLM to investigate the possibilities for formal collaboration in scientific research. I was told that recent pilot studies suggested there existed a high prevalence of hepatitis B virus (HBV) infection on certain outlying islands of French Polynesia. The islands of Rapa and Rimatara in particular (see map, figure 1), had villages where perhaps as many as 90% of the native inhabitants showed serologic evidence of HBV infection. In contrast to this, villages on several other islands had infection rates of from 30 - 60%.

In the course of discussing these IRMLM reports and reviewing the HBV literature, several especially intriguing aspects emerged. First, although high infection rates had already been documented in the South Pacific and other tropical areas, few of the other reports suggested rates as high as some of those from the IRMLM. Second, many of the studies done in the South Pacific had used relatively insensitive methods for HBV detection and had been limited to only random samplings of the populations studied. Thus, their accuracy and completeness could be brought into question. Also, none of the islands visited by the IRMLM had been included in these previous studies. Third, why did infection rates appear to vary so widely from one island to another? And finally, several hypotheses had been

put forth that genetic and other risk factors altered susceptibility to becoming chronically infected with HBV. Might these geographically, culturally and genetically isolated populations be a good model in which to study such factors?

These were the major issues and questions addressed in this study. Dr. Robert McCollum, Chairman of the Department of Epidemiology and Public Health at Yale Medical School, agreed to sponsor this project. Dr. Jacques Laigret, Director, and Dr. Francis Parc, Chief of the Clinical Immunology Section of the IRMLM in Tahiti, agreed to supervise and to provide technical assistance for the work in French Polynesia.

Two trips to French Polynesia were ultimately involved. The first, from July to September, 1978, allowed us to partially address our initial questions. I returned to Yale with serum samples from the island of Maiao as well as some which had been previously collected by the IRMLM on Rapa and Mangareva. I also obtained a genealogy on Maiao. We were able to verify a large portion of the IRMLM pilot studies, and obtained a much more complete population sampling from the island of Maiao than had been available to most other researchers in the past. But two of our initial questions still remained unanswered i.e., were there in fact widely disparate infection rates among different islands and was there evidence for genetic susceptibility to becoming chronically infected with HBV? We needed follow-up date on these populations and therefore a return trip was planned.

There were several goals for the return trip which ultimately took place from January to March 1980: 1) to attempt a more complete serum collection on the islands which had been visited originally by the IRMLM; 2) to return to Maiao to make a repeat collection which we hoped would provide essential information on the natural evolution of HBV infection in that population; 3) to

collect peripheral blood lymphocytes which could be used for more sophisticated tests of the genetic susceptibility hypothesis, and 4) to obtain genealogies from the populations on which they were lacking.

As in 1978, the vicissitudes of nature and boat captains and the generally complex logistics of an operation such as this permitted partial fulfillment of these goals. In the end I succeeded in returning to Maiao and in my absence, an IRMLM team also went, for the first time, to the island of Hiva Oa. In both locations, we were able to collect blood serum samples and lymphocytes as well as to complete genealogies. This left us, then, with serum collections from four islands and in addition, with genealogies and lymphocytes from two.

ACKNOWLEDGEMENTS

This was an audacious and complex undertaking from its inception. It was successful only because a large number of people gave extraordinarily generously of their time, energy, and expertise.

Dr. Robert McCollum, Chairman of the Department of Epidemiology and Public Health at Yale University, was my thesis advisor and the one I first approached with the idea for this project. From the beginning of our collaboration he has given unselfishly of his time and extensive knowledge of hepatitis and of the formula for successfully conducting overseas research. He has done so to an extent exceeding even my most outrageous expectations. He arranged for a major portion of the funding, handled many complex administrative matters and insured crucial support of the staff within his Department, as well as elsewhere at Yale and in Tahiti. He read and then reread the drafts of this thesis. His faith in me was unremitting in the face of many obstacles, and he was the catalyst essential for the completion of this work. I will aways be grateful.

Dr. Jacques Laigret, Director of the Institut de Recherches Médicales Louis Malardé in Tahiti, graciously welcomed me into his laboratories, supervised the arrangements for all of the field work and secured permission from the French Government for this project to proceed. His continued enthusiastic support for the day-to-day work and the alacrity with which he handled even the most delicate and complex problems was crucial to our success.

Dr. Françis Parc, Chief of the Institut Malardé's Immunology Division, was my immediate supervisor in Tahiti. His expertise enabled us to sort out many of the methodologic problems that were encountered. He provided advice and support in the planning of the field work in the form of laboratory space and equipment, supplies and transportation and the vital aid of many of his staff.

Mr. Régis Plichart of the Institut Malardé worked very closely with me in the Institut Malardé laboratories as well as in the field in both 1978 and 1980. He obtained serum specimens, interviewed subjects, did a large portion of the work of lymphocyte isolation and freezing on Maiao and was soley responsible for the lymphocyte work on Hiva Oa. He was extremely devoted to this work, and his assistance assured its success.

Mr. Roy Capper of the Yale Department of Epidemiology and Public Health performed almost all of the serologic assays for evidence of hepatitis B virus infection. His work was of such consistent high quality that the information he obtained formed the backbone upon which this entire study was constructed. He repeatedly saved the day by locating emergency relief supplies which admirably met even my most inexact specifications.

Dr. Alan Williams, post-doctoral fellow in the Yale Department of Internal Medicine, and an expert in the field of hepatitis research, aided in this project from its beginning. He gave crucial guidance in the development of the study protocol, and training in the HBV assay techniques. He personally tested our samples for the presence of the e-antigen and antibody. We have had many conversations invaluable for the clarity which they imparted to my thinking about the behavior of hepatitis viruses and he has carefully read and critiqued the thesis itself. His help has been essential.

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Ms. Allison Thurston-Palermo, formerly of the Yale Tissue Typing Laboratory, trained me in HLA-typing techniques and helped to arrange for the shipment of HLA reagents to Tahiti. She also verified interpretations of HLA typings done in 1978.

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Mrs. Fran Nankee and Mrs. Marianne Mazan handled many administrative matters, arranged for purchase and shipping of research supplies and specimens all over the world - usually with very little advance notice. I am especially grateful for their persistent efforts to decipher many of my more cryptic communications.

Drs. Michel Antoinetti and Bernard Philippe were the physicians-in-chief of the field trips to Maiao and Hiva Oa in 1980 and to Maiao in 1978 respectively. It was through their understanding and cooperation that the often cumbersome work of obtaining blood specimens and completing genealogy questionnaires was completed. In addition, they examined each of the subjects.

Mr. Manu Gay, of the Institut Malardé, provided expert assistance in all phases of the work in 1978 and in the Tahiti-based work of 1980. He interviewed a large percentage of the Maiao inhabitants and played a major role in the collection of serum specimens.

Mr. Eric Fuller, also of the Institut Malardé staff, aided in most aspects of the laboratory work in Tahiti and was a vital member of the field teams in both 1978 and 1980. His diligent and precise work helped to assure the accuracy of our results.

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from whom blood samples were obtained for use in HLA typing. In addition, he very kindly located housing for me during my 1978 stay in Tahiti.

Dr. Ian Gust of the Fairfield Hospital, Fairfield, Australia, tested some of the 1978 Maiao serum collection for evidence of both HBV and hepatitis A virus infection. In addition, he discussed many aspects of this research both with me and with Dr. McCollum.

Dr. Jay Hoofnagle of the National Institutes of Health tested many of our samples for the presence of anti-HBc.

Dr. Lacy Overby of Abbott Laboratories supplied the Abbott Auscell testing materials that enabled us to field-test serum samples for the presence of HBsAg.

Dr. Carl Cohen of the National Institutes of Health insured that we were provided with the NIH tissue typing reagents used in field tests at the Institut Malardé.

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A large number of people discussed aspects of this research with me. They have given of their time and expertise in an enthusiastic manner and thereby assured that my work was both educational and enjoyable. These individuals include Drs. Francis Black, Fred Kantor, William Credé, Wilbur Downs, Alfred Evans, Daniel Freeman, Fred Gorelick, Dorothy Horstmann, Denis Miller, Curtis Patton, Robert Shope, Byron Waksman and Steven Walter at Yale University, and Drs. Françis Louis, Gaston Pichon, Charles Tetaria and Paul Robert Thomas in Tahiti.

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Mrs. Amy Mangini typed this thesis.

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INTRODUCTION

The goals of this research project were twofold: one, to study the epidemiology of hepatitis B virus (HBV) infection in native tropical island populations and two, to test the hypothesis that the development of the HBsAg-positive chronic carrier state as the host's response to infection with HBV is subject to genetic determinents.

HBV epidemiology has been studied throughout the world and some of the highest infection rates have been found in persons living in tropical climates. With few exceptions, practical constraints necessitated that most of the studies conducted thus far were based on cross-sectional samples which may or may not have been representative of the total population at large. However, in spite of such uncertainties, the majority of authors reported very large proportions of apparently asymptomatic carriers of HBV virus in such tropical populations (18, 39-43, 81, 121). Such a phenomenon has been reported only rarely (12, 114, 115) elsewhere.

This project was initially conceived as an attempt to either confirm or refute these past observations in the more suitable model of an isolated population in which major sample biases could be eliminated by testing nearly all members of the community for evidence of HBV infection. In so doing, the more sensitive methods would be employed to detect past, acute or chronic forms of infection.

Secondly, geographically and potentially genetically isolated populations constituted an excellent experimental model in which to address the question of genetic susceptibility to developing chronic HBV infection. When most people

are infected by HBV they have an acute and relatively short-lived infection which resolves as the host synthesizes antibodies to the infecting virus. However, a smaller number of people seem not to mount such an antibody response and remain persistently infected with HBV i.e., chronic carriers of HBsAg. If there is indeed a genetic basis to the development of the chronic carrier state, one might see the clearest evidence of its operation by studying a population in which a large proportion of individuals are infected with HBV and which is isolated enough that the gene pool is relatively stable over at least several generations. Persons living on remote tropical islands who travel infrequently and who receive few vistors seemed to be just such a population - assuming one could practically gain access to them.

Past studies of island populations have used as their measure of a genetic influence on the development of the carrier state the frequency of clustering of chronic carriers within families (7,83). Conclusions based on such data may well reflect the operation of other than genetic influences. The next level of sophistication is to look for patterns of transmission and acquisition of HBV infection in detailed population pedigrees. This has been reported on a limited basis (11, 80, 101, 117, 138, 145), but only in isolated families not part of these tropical populations. However, the method of pedigree analysis, when used alone, is still of limited power compared to when it is used with more recently developed methods of genetic analysis such as HLA typing.

HLA typing is based on a biologic assay of the gene products directly involved in human immune system regulation. Thus, we hoped to include in this study not only seroepidemiologic documentation of HBV infection in isolated tropical populations, but also a genetic analysis of the development of the chronic carrier state based on both pedigree analysis and HLA typing.

In the end, we were able to address all of our original questions, although all of the results cannot be presented and discussed in this thesis. The sero-epidemiology of HBV infection in native inhabitants of four islands in French Polynesia is reported here. In addition, longitudinal serologic data relating to the development of the chronic carrier state, as well as a genetic analysis based on pedigree data, are reported for one of these islands. The complex nature of the HLA analysis and the length of time required for its completion make it necessary to present those data in a separate report.

HEPATITIS B VIRUS STRUCTURE AND SEROLOGY

Hepatitis B virus has a unique structure among viruses known to cause disease in humans. It has a 43 nm spherical shape (also known as the Dane particle) with a 27 nm inner core containing a unique combination of double-stranded circular DNA, and a specific DNA polymerase. There are three different major antigenic determinants: the surface antigen (HBsAg), the core antigen (HBcAg) and the e-antigen (HBeAg). HBsAg reactivity is found on the surface of three particles: the Dane particle and the 22 nm spherical and filamentous forms which result from excess hepatocyte protein coat synthesis in the infected host. HBcAg reactivity is found on the 27 nm inner core, and HBeAg reactivity also appears to be associated with the Dane particle core (89). HBsAg has a group-specific determinent "a'" and two mutually exclusive subtupe pairs, "d" or "y" and "w" or "r". HBsAg is therefore classified as subtype adw, adr, ayw or ayr (69).

Each of the HBV antigens has a corresponding antibody which may be synthesized by the host in response to infection.

SEROLOGIC RESPONSES TO HBV INFECTION

Circulating HBsAg has been detected as early as six days after parenteral HBV exposure, but is usually first detectable starting from four to six weeks prior to onset of the clinical symptoms of acute infection (66) and persists for a period of from one to four months thereafter (see figure 2). However, studies of experimental inoculation have shown a very wide range of duration of HBsAg positivity ranging from one day to many years (10, 63). A detailed discussion of the development of the HBsAg-positive chronic carrier state will be found in the section on epidemiology. Figure 3 shows an example of the evolution of serologic markers of infection in a chronic carrier.

Although HBsAg appears to be intimately related to the infective, disease transmitting particle of HBV (105), it has been shown that blood (or other tissue) which does not contain measurable HBsAg can also produce HBV infection (35). This phenomenon may be due to the insensitivity of a particular assay, but may also result from having assayed the infective material at a time in the natural course of HBV infection when HBsAg production is declining in proportion to increasing synthesis of anti-HBs (precipitating antibody) i.e., sampling is done during a relative steady-state when the circulating amounts of antigen and antibody are balanced and bound in such a way that no net antigen or antibody is measurable. This is represented in figure 2 as occurring at about for to five months after infection.

The core of the Dane particle has its own antigenic specificity, HBcAg. Although HBcAg positivity during clinical infection is relatively short-lived compared to that of anti-HBc (126), HBcAg appears earlier in the disease course during the interval of combined HBsAg and anti-HBs negativity (14, 55, 141) and thus may provide the only evidence of HBV infection in otherwise seronegative serum (62, 91). This could in part explain the cases of HBV transmission by transfusion of supposedly HBsAg negative blood (54) mentioned above. For these reasons, anti-HBc determinations are assuming an increasingly prominent role in studies of HBV epidemiology.

The functional significance of the HBeAg/anti-HBe system is discussed in the section on HBV epidemiology. It is currently considered important because of its potential value as a predictor of the infectivity of HBsAg-positive material as well as of the duration and resolution of infection. The time course of HBeAg and anti-HBe appearance is indicated in figure 2. Serum HBeAg is found only when HBsAg is present (14, 92, 118) and may be associated with unusually high

titers (by RPHA) of HBsAg (93, 118). In a situation analagous to that suggested for HBsAg and anti-HBs, there may be a transient period of combined HBeAg/anti-HBe seronegativity when no net excess of either is present. This is depicted (figure 2) to occur starting at about three and a half months post infection.

In a discussion of data collected from a large study of experimentally infected volunteer male prison inmates, Hoofnagle et al. summarized five patterns of serologic response after exposure to HBV (53). These are indicated in Table 2. Because this thesis is particularly concerned with the development of the chronic carrier state, an example of the serologic responses to HBV infection in a patient who went on to become a chronic carrier is shown in figure 3.

Serologic assays

Several different techniques, varying considerably in both sensitivity and specificity,are available and have been employed in the past as assays for each of the three major HBV antigen/antibody systems as well as for subtyping. These are summarized in table 1. Of note is that there are dramatic differences in sensitivity between the assays commonly employed today (the RIA and PHA or RPHA tests) for HBsAg and anti-HBs and those utilized in much of the early research on HBV epidemiology.

EPIDEMIOLOGY OF HBV INFECTION

Worldwide Prevalence of HBV Infection*

Studies of hepatitis B prevalence have been conducted in most parts of the world, representing a wide variety of communities and climates.

Measurement of HBsAg has been the most often employed assay for evidence of "HBV infection". A committee of the World Health Organization has defined the HBsAg carrier state (based on a survey of longitudinal studies) as "the presence [in the blood] of HBsAg for more than six months" (3). The number of human carriers was estimated to be 120 million in 1977 and HBsAg prevalence (but not carrier state) in "apparently healthy" adults varied from 0.1% in Europe, North America and Australia to 15% and more in some tropical countries. In countries where HBV infection was uncommon the highest prevalence of HBsAg was found in the 20-40 year old age group but, where common, the highest prevalence was in 4-8 year old children (3).

A 1978 review by Szmuness et al. (126) gave worldwide HBsAg prevalence figures of less than 0.5% in the U.S. and Western Europe, 1-2% in South America and Southern Europe, 3-5% in North Africa and the U.S.S.R. and 6-10% or higher in some parts of Africa and Southeast Asia. It also suggested a combined infection rate (persons with HBsAg plus those with anti-HBs and anti-HBc) of 7-10% in the U.S. and 60-80% in Southeast Asia or Africa.

It should be pointed out that the figures reported above are average values derived from surveys of large populations and that smaller, special population groups may have infection rates very different from those found in the population at large e.g., drug addicts or institutionalized patients. This probably reflects a combination of factors including increased frequency of occurrence of certai

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* Many of the references included in this section were first listed in the Hepatitis Knowledge Base (July 1980 version), kindly supplied by the Lister Hill National Center for Biomedical Communications.

modes of HBV transmission or increased susceptibility of the population to infection due either to genetic or to environmental factors which alter the host immune response.

Modes of transmission

Most authors tend to discuss HBV transmission in terms of "parenteral" or "nonparenteral" routes. As discussed by Mosley in a recent commentary (85), the term parenteral has become synonymous with "percutaneous". Certain proposed modes of transmission such as fecal-oral, vertical, sexual, respiratory, and the catch-all, "close association", have been lumped together under the term "nonparenteral", which in turn has become synonymous with "nonpercutaneous". Defining parenteral "as applicable to anything ...taken into the body in a manner other than through the digestive canal", Mosley goes on to say that there are two good reasons to abandon this parenteral vs. nonparenteral disctinction: 1) several of the routes of infection classified as nonparenteral i.e., oral, sexual or respiratory, may in fact be parenteral and 2) the nonpercutaneous "nonparenteral" routes such as sexual contact and vertical transmission may well prove to be far more significant epidemiologically than the percutaneous "parenteral" routes. Therefore, especially since the epidemiologic significance of these nonpercutaneous routes is very different worldwide, it may be inappropriate to lump them together under a single label.

A somewhat different conceptualization has been advocated by Szmuness et al. (126). They proposed that variations in the mode of HBV transmission are "mediated by a large variety of risk factors". Three major categories of risk factors provide a framework within which mechanisms of transmission may be classified: (A) Enhanced probability of exposure, (B) Enhanced probability of HBsAg persistence and (C) Both enhanced exposure and persistence. The details of their schema will be

found in table 3 and may be helpful in understanding the following discussion.

Mechanisms

1) Direct parenteral (intravenous or percutaneous) inoculation

There are many studies documenting intravenous HBV transmission e.g., in hemodialysis patients (79), and recipients of blood and blood products (11, 88), via contaminated needles in drug abusers (22) or by accidental needle stick in medical personnel (79). There is even the suggestion that HBV-containing material may penetrate bare feet in some areas, thereby transmitting infection (131). The human bite may also effect HBV transmission (47, 77, 87) as might that of certain arthropods. This latter mechanism is of potentially greater concern in tropical climates where arthropods are especially numerous. Arthropod transmission of HBV from mosquitoes, bedbugs and cockroaches has been proposed (73, 86, 90, 95, 103, 140, 143) but its real ecological significance is unclear.

2) Contact transmission

This is perhaps the most controversial form of HBV transmission and there are many conflicting studies in the literature. The major proposed routes are sexual, oral (via saliva or ingestion of HBsAg-positive material) or fecal-oral.

Indirect evidence in favor of contact transmission comes from studies of HAV outbreaks in which patients who clinically and serologically appeared to have hepatitis A were found also to be HBV-positive (23, 36, 49, 142). Other findings which suggest the operation of contact transmission include the spread of HBV by the fecal-oral (64) or other "nonparenteral" routes (50, 120, 125) in institutions for the retarded, the finding of high proportions of HBV infection among persons living in the same households as a known HBsAg carrier (100,113, 122), the occurrence of an area-wide epidemic and an outbreak among children in the absence of

evidence for percutaneous transmission (135), and the occurrence of outbreaks under circumstances where a percutaneous mechanism seemed unlikely to operate (31, 133). Oral-oral or fecal-oral (136) as well as respiratory transmission (5, 136) have also been postulated to occur in other circumstances.

Early evidence against contact transmission of HBV came from studies in which oral or nasal administration of presumed HBV positive material failed to cause clinical hepatitis in volunteer subjects (46, 76). However, it is known that the gut contains HBsAg inactivating enzymes (38, 102).

A study of hepatitis B occurrence in contacts of persons receiving HBV-contaminated yellow fever virus vaccine showed few secondary cases (28, 99) and a plasmapherisis-associated epidemic in prisoners likewise showed few clinical cases among the secondary contacts of persons receiving the contaminated blood products (59, 108). One must be cautious in interpreting these latter studies because, although they may have accurately reported the incidence of clinical hepatitis, they relied on the appearance of clinical signs and did not include the occurrence of inapparent HBV infection.

The studies of possible venereal transmission of HBV have yielded inconsistent results. For example, some venereal disease clinic patients who professed a wide range of sexual contacts had an increased frequency of infection (30, 58) as did presumably promiscuous homosexual men (27, 124), although homosexual women did not(124). One study of prostitutes showed they had no greater frequency of HBV infection than did chaste nuns (18), whereas a study comparing prostitutes to age-matched female blood donors or nuns (29) and another study of prostitutes alone (96) did show higher than expected prevalence of HBV infection among the prostitutes.

3) Vertical transmission from mother to infant

Vertical transmission probably plays a significant role in HBV propagation,

especially in populations where very high carrier and infection rates exist. It is conceivable that infection occurs either in utero (via HBsAg positive amniotic fluid or transplacentally), at the time of birth (secondary to contact with antigen-positive vaginal secretions or maternal blood) or, in the perinatal period by several possible routes.

Evidence in favor of in utero transmission includes the finding of HBsAg-positive amniotic fluid at 37 weeks gestation (71) or at the time of birth (by Caesarian section) (34), and the finding of antigen-positive cord blood (presumably with HBsAg having been synthesized by the fetus after transplacental infection) in both antigen-positive (20, 71, 94, 110) and negative (110) mothers. For example, in one study 70% of babies became HBsAg-positive after birth when their mothers had been infected during the third trimester whereas only 6% became positive when infection had occurred during the first or second trimester of pregnancy (110). Indeed, one mother who had been infected at six weeks gestation and who had subsequently become antigen-negative, nevertheless delivered a baby whose cord blood contained HBsAg (110). Dane particles have been observed in cord blood known to be positive (by RIA) for HBsAg (20).

Both anti-HBc (110) and anti-HBs (94) can cross the placenta and there is indirect evidence that anti-HBs can cross even more readily than HBsAg (94).

That transmission can occur at the time of birth is suggested by the finding of HBsAg-containing vaginal secretions in the mother and HBsAg in the gastric aspirates from newborns sampled at the time of delivery(71). In addition, it is obvious that the fetus may come in contact with HBsAg positive maternal blood during delivery.

Finally, breast milk has been shown to contain HBsAg (13, 71, 74) although its role in transmission of infection is uncertain (13, 25, 71).

Epidemiological surveys do not directly elucidate the mechanisms of transmission, but they have suggested a significant role for vertical transmission of HBV infection.

As is discussed elsewhere, infection prevalence is often highest in the younger age groups and early acquisition has been interpreted as indirect evidence of the operation of vertical transmission since in many societies the infants spend a significant portion of their time in close maternal contact. In a family study on Santa Cruz Island in the South Pacific (83), Mazzur found a clustering of HBsAg and anti-HBs positive children only in families with an HBsAg positive mother (21%). This suggested to her that vertical transmission is at least one important mode of spread of infection. Other studies have also found that disproportionately high percentages of infants born to HBsAg carrier mothers develop antigenemia in the first one to two years of life (13, 14, 25, 71).

The role of the e-antigen/antibody system in vertical transmission has been extensively studied in the orient. One study of 23 HBsAg-positive (by immune adherence hemagglutination) Japanese mothers found that 100% (10/10) of babies born to those mothers who were HBeAg-positive developed HBsAg during the first year of life. In contrast, none of the babies born to anti-HBe positive mothers did developed HBsAg. Of the remaining six babies born to mothers both e and anti-e negative, two developed HBsAg and four did not (93). Another study of HBsAg-positive Chinese women (using RIA) found that 85% (17/20) of babies born to HBeAg-positive mothers developed HBsAg and also that 31% (13/42) of babies born to HBeAg-negative mothers developed HBsAg. Only one mother in this study had anti-HBe (14).

Age and Sex Distribution

The literature contains a large number of reports of age and sex distribution

of HBsAg. The initial and now classic work in this area is that of Blumberg (17). His conclusion was that HBsAg occurs more frequently in males less than 20 years old. There have been a multitude of other studies which have tended to confirm the trend for increased frequency of HBsAg in young males, although the sex difference has not always been significant nor the age group the same. The mechanisms accounting for these observations are highly debated and may reflect the interplay of cultural (82), genetic (16), or other factors. For example, sex-specific behaviors and occupations or sex-linked immunogenetic factors (a detailed discussion will be found in the next section) may play a role. This is true in some of the remote tropical populations which have been studied (6, 17, 26, 39-43, 81, 82, 123, 141), as well as populations in temperate (18, 37, 107, 119) and colder (8, 12, 114, 115) climates. Table 4 summarizes some representative data. Almost all of these studies have incorporated simultaneous anti-HBs measurement and it is generally found that anti-HBs prevalence increases gradually with age.

Anti-HBc distribution has received more limited study but may more accurately reflect HBV infection rates than either HBsAg or anti-HBs prevalence (whether used singly or in combination). A recent study (141) of five different South Pacific populations showed marked heterogeneity in HBV infection rates as determined by retrospective assays for anti-HBc in sera from the late 1950s and early 1960s. There were generally no significant differences in the prevalence of HBV infection in males or females, but the age-specific prevalence varied tremendously between Melanesian, Micronesian and Polynesian populations and in particular between two Polynesian islands (Tahiti and American Samoa) and two islands within French Polynesia (Tahiti and Bora Bora) itself. These data are also summarized in Table 4. Another study in Greenland reported a fairly good

correlation of anti-HBc with HBsAg positivity in two populations which showed a 1.5:1 male:female ratio but no significant age-specific differences in HBsAg frequency (114). A third study in tropical Africa showed a gradual increase of anti-HBc with subject age, but no differences in sex distribution were found (26).

Age-specific prevalence studies suggest that HBeAg decreases while anti-HBe increases with age among HBsAg-positive subjects and that HBeAg prevalence is highly correlated with a high titer of HBsAg (84, 92, 114). Only the relatively insensitive immunodiffusion assay was available for these studies. A newly developed commercially available RIA will no doubt soon allow for a much more thorough investigation of the role of the e/anti-e system in HBV infections.

Chronic Carrier State

In the strictest terms cited earlier, the chronic carrier state is defined by serologic evidence of HBsAg positivity on two measurements separated in time by at least six months. Some studies have measured incidence of HBV infection to most accurately establish HBsAg carrier rates, but for obvious practical reasons, there are relatively few population studies with such multiple samplings. Rather, single point prevalence studies are commonly done, especially in the less accessible populations. As will be discussed more fully below, incidence studies which have shown relatively high HBsAg carrier rates (in the 10-20% range) have invariably found nearly the same age-specific rates on repeat samplings. Therefore, the empiric assumption is made, and indeed this has become the accepted practice, that high HBsAg point prevalence rates also accurately reflect HBsAg carrier rates.

When HBV infection leads to the development of the chronic carrier state, HBsAg usually rises to a high titer over a period of several weeks and then

remains elevated due to persistent virus replication (53). The time of onset of post-infection viremia can vary and to some extent has been a function of the detection method employed. Krugman et al. (66) point out that the minimum incubation period was thought to be 60 days in the late 1940's when appearance of jaundice was used as a marker of infection, 41 days in the 1950's with transaminase enzyme elevations as a marker, 29 days in the early 1970's using complement fixation to measure HBsAg and now 5-7 days using a radioimmunoassay to measure viremia.

In the typical acute HBV infection, surface antigenemia rarely persists for more than four months (66). Persistence for longer than this usually indicates the development of the chronic carrier state, although there are reports of loss of HBsAg and subsequent appearance of anti-HBs after periods of from six months to many years (63, 104, 116, 120, 137, 146). This may reflect the operation of several factors including a natural decline in antigen to undectable levels with advancing age (120), a differential morbidity/mortality due to HBV infection (137) or genetic factors (see below). For example, one report of a group of persistent carriers infected early in life suggested that HBsAg clearance could occur after 20-30 years of positivity (126). Of course, population studies which do not follow the same individuals over time do not directly measure the duration of the carrier state. The epidemiologic grounds for inferring such information are discussed elsewhere.

If anti-HBs is made at all in chronic HBsAg carriers, it is probably in such small quantities as to be entirely complexed with circulating antigen (53). In rare cases a heterotypic antibody response occurs i.e.,directed against a different antigen subtype. This may be an antibody response to an earlier infection while virus of a different subtype has infected secondarily (or vice versa) and

has been observed in both nonhuman primates (128) and in man (68, 127).

Early studies of anti-HBc (51, 62, 65) found that high titers (detected by complement fixation) were seen in acute HBV infections and then declined to undetectable levels within about one year, whereas they remained elevated in chronic carriers. It was suggested that high anti-HBc titers indicated continuing virus replication and therefore were also indicative of infectivty. More recent work has shown, however, that anti-HBc (as measured by RIA) can persist at low levels after recovery from infection, even when anti-HBs is present; thus it was suggested that persistent anti-HBc could indicate immunity rather than infectivity (66). Along these lines, it has been shown that two classes of anti-HBc molecules exist and that IgM-anti-HBc may be a marker of ongoing virus replication and infectivity while IgG-anti-HBc may be present for many years in persons with completely resolved infection (91).

Evidence is accumulating that the e-antigen/antibody system may be associated with factors which determine the infectivity of HBsAg-positive material as well as the duration or outcome ofinfection. The presence of HBeAg during acute hepatitis may be predictive of development of the chronic carrier state in some subjects (9), but at least one study has found no correlation at all (129). There is one report from Polynesia that 38% (21/56) of HBsAg carriers also had HBeAg whereas 4% (2/56) had anti-HBe (40).

The Genetic Hypothesis of Susceptibility to HBV Infection

A tremendous amount of scientific controversy and fruitful research were generated by Blumberg in 1969 when he proposed that susceptibility to persistent infection with HBV is inherited as an autosomal recessive trait (16). His original hypothesis was based on family studies of tropical populations living in

two separate island communities where he found a remarkably close fit between predicted and observed frequencies of HBsAg as measured by an immunodiffusion technique.

His hypothesis, which predicted that only people homozygous for the susceptibility gene should become chronic carriers, was repeatedly tested in other populations. Early support came from a major study of some 700 Thai's living in a Bangkok housing development (39) as well as from a study of caucasians living in Sardinia (21).

The 1971 Thai study is worth going into in some detail. The population was picked to be a representative and random sample. Blood specimens were obtained at five and at nine month intervals and tested for HBsAg by both immunoelectroosmophoresis (IEOP) and the more sensitive RIA, and for anti-HBs by IEOP and PHA. Only 1/679 (0.1%) of the subjects tested developed clinical hepatitis. Serum glutamic oxaloacetic transaminase levels were virtually identical for those persons positive or negative for anti-HBs and generally not elevated (81%), to only slightly higher (5%), but still normal in those positive for HBsAg. Of the nearly 700 original subjects, 523 were followed with paired serum determinations and of these, 37/39 (95%) who had been HBsAg-positive originally remained positive over the nine month period. Age-adjusted HBsAg prevalence rates were 10% males, 7% female and 8% combined, and were significantly higher in males than in females of ages 10-29 years. Anti-HBs prevalence was higher in males up to age 60 years with overall prevalences of 52% in males, 42% in females and 46% combined. When HBsAg and anti-HBs positives were taken together as an overall measure of HBV prevalence (anti-HBc was not measured), 21% of children 1-4 years old were infected and there was a steady rise in infection rate to a level of 68% in the greater than 40 year old

age group. Combined incidence of HBV infection (those subjects who acquired either HBsAg or anti-HBs during the nine month study period, and who had been negative for both at the onset) was 13% and was also greatest in the 1-4 year old age group. Finally, by adding to those who had developed new HBsAg or anti-HBs, the number of individuals who showed serologic evidence of reinfection, as defined by at least a four-fold increase over baseline anti-HBs titer (such anemnestic responses have been documented elsewhere (24, 126), the authors calculated that as much as 19% of the entire population could have been infected during the nine month study period.

From these data they concluded that (1) since two-thirds of those infected showed evidence of re-infection during the study period, it was probable that nearly everyone in the community would be infected one or more times in his lifetime and (2) since some people in every age group lost serologic evidence of infection, overall HBV prevalence could never reach 100%. It plateaued at 67% in this population.

Next, they found no significant differences in infection rates as a function of likely risk factors, i.e., a history of hospitalizations, medical infections, dental work, tatooing, blood donations, blood transfusions or jaundice. Since they had concluded that a large majority of HBsAg-positive subjects were probably long-term carriers, they were surprised at the lack of increased relative risk in these carriers, although they did point out that poor sanitation, crowding and "frequent intimate contact" were the rule, and that potential arthropod vectors were widely prevalent.

All in all, these findings suggested to the authors that genetic susceptibility to HBV infection might play a role in the development of the chronic carrier state.

Furthermore, there was a significantly higher prevalence of HBsAg among children in families in which the mother was HBsAg-positive. They suggested this might be due to more prolonged and intimate contact between mothers and children (at least at very young ages) which afforded greater opportunity for the child to become infected. In the end they decided their findings were most consistent with the hypothesis that genetic susceptibility (although they did not specify Blumberg's autosomal recessive mode) to HBV infection operates to favor the development of the HBsAg-positive chronic carrier state.

A 1973 study of HBV prevalence in Senegal (121) also considered the genetic hypothesis in two separate groups of children between five months and 14 years of age and in servicemen, although only the data collected on children are discussed. Among 1430 children, 16% of males and 11% of females (or 13% overall) were HBsAg-positive by agar gel immunodiffusion. Some 804 children were retested after six months, including 105 of the original 186 who were HBsAg-positive and 99/105 (94%) remained HBsAg-positive while 18 (1%) additional children had acquired HBsAg. In contrast to the hypothesis proposed in the Thailand study, these authors suggested that sanitary, socioeconomic, other environmental factors and perhaps vertical transmission played the major roles in causing the high carrier rates. They could not account for the observed sex differences (HBsAg 50% higher in males), although the pattern of higher HBsAg carrier rates among males noted in many population studies (8, 17, 37, 123) appeared to be environmentally and culturally induced on one South Pacific island (82).

Bänffer et al. (7) studied ethnically distinct groups of Indians, Creoles and Indonesians living in the capital city of the Republic of Surinam in 1974. Although HBsAg prevalence was 19% in the Indonesians vs. 3-8% in the other groups, the authors postulated that family clustering of carriers (which was "suggested"

by their data) may have been due to a greater exposure risk rather than to a genetic susceptibility. Since it appeared that the Indonesians were among the last groups to move from their native homeland to the city, perhaps they brought with them a greater HBsAg prevalence which was in the process of decreasing to the levels of the surrounding populations. In other words, their particular household environment/life-style, although beginning to conform to new surroundings, still placed them at increased risk for becoming chronic carriers.

Early evidence against this hypothesis was the finding in a Sardinian family that only 2/7 children of an HBsAg-positive mother and father (presumably both homozygotes) were HBsAg positive by immunodiffusion assay (101). However, some doubt was raised as to whether all of the children had in fact been exposed to HBV. In 1974 Vyas studied a Chinese family (138). The parents both remained HBsAg-positive (by RIA) over a six month period whereas two children were both antigen and antibody negative and the third child anti-HBs positive during the same period. Lederberg (70) pointed out at the same time that family clustering of HBsAg carriers could probably be explained on the basis of spread through prolonged close personal contact without invoking a genetic basis. Key family studies published in 1976 (80, 117, 145) and 1977 (83) also had results incompatible with the autosomal recessive hypothesis.

In 1975, Szmuness et al. published a paper which went far towards clarifying the environmental vs. genetic influences debate (119). They studied 751 New York City families of blood donors and found that in families with an index-donor HBsAg-positive, overall prevalence of HBV infection was ten times as high as in control families of the same ethnic background. Similarly, in families with an anti-HBs-positive index donor, prevalence was two to three times as high as in controls. Genetic segregation analysis suggested an excellent fit with

the autosomal recessive hypothesis and in two of three families with both parents HBsAg-positive, all progeny were also antigen positive.

Their comments on the significance of their own and others' (11, 80, 101, 117, 138, 145) pedigree analyses, even though published before some of these other studies, bear repeating here. First, failure to detect antigen in progeny of putatively homozygous parents may reflect the inability of the testing method employed to detect low levels of antigen, the fact that HBsAg carriage is not always life long, or the possibility that susceptible progeny have not yet been infected (120). Even if the homozygous recessive hypothesis is incorrect, they state, the individual's response to HBV infection may be mediated by "some unidentified polygenic inheritance". The HLA system is certainly a good candidate to fill this role.

The Histocompatibility System

Chronic carriers only rarely show overt clinical signs of hepatitis (4, 10, 53) and they often show no or only mild elevations of transaminase enzyme levels (60, 61). It is widely believed that these observations reflect the operation of a host immune system tolerant to HBsAg (109, 138) secondary to abnormalities of white blood cell function. Although there is at least one report of neutrophil dysfunction in chronic carriers of HBsAg (134), most discussion by far has centered around alterations in lymphocyte-macrophage handling of HBV as leading to the development of persistent antigenemia. The immunologic function of these cells is thought to be largely under the control of the polygenic HLA system.

The HLA system is the major histocompatibility complex in man and is believed to control, or at least to be in close genetic linkage with the genes that control, immune system function. HLA genes are multiallelic and code for different proteins found on the surface of all nucleated cells and also platelets (78). Interactions between immune system cells and host or foreign antigens (for example, HBsAg) are

thought to be in large part keyed to these cell surface HLA antigens.

The exact mechanisms which are postulated to lead to increased disease susceptibility or the development of the chronic HBsAg carrier state, are beyond the scope of this thesis. However, HLA associations in patients with hepatitis B infection have been studied and are listed here for HLA loci A and B. Among persons who are HBsAg positive chronic carriers there are reports of an increased frequency of HLA loci A11 (111), A3/B19 (132), Bw17/Bw27 (44), Bw15 and B8(56). However, one study of chronic carriers found a decreased frequency of A2/Bw15 (19). In chronic carrier hemodialysis patients, positive associations have been reported between loci A11 (111), B8 (98) and Bw15/Bw17/Bw35 (48). One study found no associations (57).

Many population studies have confirmed the observation first made by Blumberg in 1969 (17) that HBsAg occurs more frequently among males. In a more recent study of HBsAg-positive hemodialysis patients in which Blumberg participated (75), the suggestion was made that y chromosome-linked genes, perhaps in concert with HLA histocompatibility antigens, influence the male host response to HBV infection.

Rapid progress is being made in HLA typing technology so that significantly more specific reagents are available now than were used in some of the studies quoted here. At present, there are still no universally agreed upon associations between HLA type and susceptibility to, or outcome of, HBV infection.

METHODS

Blood samples were collected from Maiao and Hiva Oa during two separate trips to French Polynesia, in July of 1978 and January of 1980. The purpose each time was to obtain and preserve, from each subject, blood serum for HBV serology, peripheral blood lymphocytes (for HLA typing), to perform physical examinations and to compile a genealogy of the entire population. Blood samples from Rapa and Mangareva had been collected previously by IRMLM personnel. In both years, the research team was comprised of a physician, nurse, and two technicians from the IRMLM. I was present for the field work on Maiao in 1978 and 1980. The Hiva Oa specimens were collected by the IRMLM team. Transportation of personnel and equipment was accomplished using various combinations of boats and airplanes as were necessary to assure adequate collection times and the preservation of the specimens. In total, 792 blood specimens were obtained.

Each island to be visited was contacted by radio in advance of arrival and the nature of the visit explained. This research was conducted within the context of the annual or biannual medical missions to outlying islands which are conducted by the staff of the IRMLM and the Public Health Department in Tahiti. Thus venipuncture and physical examinations would have been performed as a matter of routine and were readily accepted by the populations. Yale University Human Investigation Committee approval of this design was obtained beforehand.

Data were collected over 5-10 day periods which varied from island to island.

In 1978, trips were made to the islands of Rapa, Mangareva and Maiao. At that time blood samples were collected on all three islands and a geneaology completed on Maiao. Lymphocytes for HLA typing were not collected.

In 1980, a return trip to Maiao was made and a repeat serum collection and lymphocytes obtained. This repeat serum collection was intended to provide information on acquisition of HBV infection since the 1978 baseline collection.

Similarly, blood serum, lymphocytes and a genealogy were obtained in the village of Atuona on the island of Hiva Oa.

Serum Collection

Blood samples were collected under sterile conditions by standard venipuncture techniques. Approximately 10 - 15 ml of blood was placed into dry tubes and allowed to clot for serum separation. Separated serum was stored in labelled tubes at +4°C while in the field locations (usually for several days) and then frozen at -20°C once returned to the IRMLM. They were eventually shipped on dry ice to Yale University and kept at -20°C thereafter.

Genealogies

Genealogy data were acquired by multiple interviews with each family in a given village. This was accomplished with the help of several IRMLM technicians fluent in the local Polynesian dialects and we employed a specially designed questionnaire (see example in figure 4) supplied by Dr. Kenneth Kidd of the Department of Human Genetics at Yale. Data were subsequently transcribed for pedigree analysis.

HBV Serology

Serum samples were analyzed at Yale University. All sera were first tested for the presence of anti-HBs by reversed passive hemagglutination (RPHA, commercially available from Abbott Laboratories). HBsAg-positive samples were further tested for HBeAg and anti-HBe by agar gel immunodiffusion (69). Surface antigen/antibody negative samples were tested for anti-HBc by radioimmunoassay (CORAB, commercially available from Abbott Laboratories).

Study Populations

All of the subjects were native-born inhabitants of the islands under study. They are principally a Polynesian population but they have been intermittently visited by increasing numbers of Eurasians since the 18th century and there has been an unknown amount of interbreeding.

Geographical Description

French Polynesia is located in an area more than 6000 km from the nearest continent. It is composed of some 120 islands loosely grouped into five archipelagos located between 8° - 28° south latitude and 134° - 155° west longitude. Alaska is 7500 km and Hawaii 4400 km to the north, Antartica 5500 km to the south, and Peru 7800 km to the east. Micronesia, Melanesia, Indonesia and finally Australia (5300 km) lie to the West. A map of French Polynesia is shown in Figure 1. It is 7700 km and a ten hour jet ride from Los Angeles to Tahiti.

The islands are composed of two principle types, high volcanic and low coral atolls. Atolls are formed by the deposition of calcium carbonate coral skeletons onto a substrate of submerged volcanic rock. They are arbitrarily defined as being less than seven meters above sea level. Many islands therefore have both volcanic and coral elements.

The Society archipelago is a group of fourteen mostly volcanic islands, the windward islands being Tahiti, Maiao, Moorea, Maheita and Tetiaroa, and the leeward islands Huahine, Raiatea, Tahaa, Bora Bora, Maupiti, Tupai, Mopelia, Scilly and Bellinghausen.

The Tuamotu archipelago is composed of 76 atolls, of which Rangiroa is the largest and best known.

The Gambier archipelago is really the southwesterly prolongation of the Tuamotu's and is made up of a large atoll named Temoe surrounded by eleven main islands including Mangareva and Aukena.

The Austral archipelago is composed of seven islands of which the five principle volcanic islands are Rimatara, Rurutu, Tubuai, Raevavae, and Rapa.

Finally, the northernmost Marquesas archipelago is comprised of ten volcanic islands, including a northern group of which the main island is Nuka Hiva and a more southern group with Hiva Oa the main island (106).

As can be deduced from study of the attached map of French Polynesia, access to many islands is limited by the great distances involved. There are few airports and none of the islands in this study had hotels. Only Mangareva had any permanent foreign residents. These islands only rarely receive outside visitors and the islanders themselves travel even more infrequently.

Maiao, one of the society islands group, is a small mountainous island located 185 km from Tahiti: It measures about 5 x 5 km, with its tallest point 150 m, and has a central mountain ridge bordered by swampy flatlands and an outer ring of coral sand. It is entirely surrounded by a barrier reef except for two small passes. There is a single village of some 200-225 inhabitants living in about 35 houses.

Hiva Oa is a mountainous island in the Marquesas group, some 1200 km northeast of Tahiti. It is famous as the setting for Melville's Typee and as the burial site of Paul Gauguin. Atuona village is located in an isolated valley called Pua mau. It consists of about 170 inhabitants living in a cluster of 35 houses.

Mangareva is a high island 1450 km southeast of Tahiti. It is a large island surrounded by a number of smaller islands which are actually raised portions of the barrier reef. The village of Rikitea has some 468 inhabitants living in 95 houses.

Rapa is also a high island and perhaps the most isolated of all the inhabited islands of French Polynesia, located 1100km southeast of Tahiti. There are two villages, Ahurei and Area, located close to one another with a population of 398 split between them and living in 104 houses.

FACILITIES

The work was carried out in two stages, sample collection and then laboratory testing. The sample collection in French Polynesia was done with the cooperation of the Institut de Recherches Médicales Louis Malardé (IRMLM) in Papeete, Tahiti. Subsequent work was done at Yale University.

The IRMLM is the center of scientific research in French Polynesia and indeed one of the preeminent centers in all of the South Pacific. Its main laboratories and offices are located in the city of Papeete on the island of Tahiti, but it also has an insectorium, marine stations and other facilities located elsewhere on Tahiti and on other islands. The IRMLM holds outpatient clinics both at the Institut itself and in liason with the Public Health Service, on almost all of the inhabited islands of French Polynesia.

The staff is composed of the Director, the Chiefs of the three main divisions (Clinical Immunology, Marine Biochemistry and Arbovirus Research), a physician in charge of clinical activities and a large number of laboratory technicians, nurses and other personnel.

The HBV serology and all data analyses were carried out in the Department of Epidemiology and Public Health of the Yale University School of Medicine.

RESULTS

In 1978, blood samples from 151 individuals living on the islands of Maiao, Rapa and Mangareva were tested for HBsAg, anti-HBs or anti-HBc. In 1980, 156 individuals on Maiao were tested in addition to 120 persons living on Hiva Oa.

The relative proportion of the total population represented by the individuals tested is shown in Table 5. In the following discussion, the 1978 Maiao samples provide baseline data and thus the 1980 collection is used only for comparison purposes in deriving measures of HBsAg carrier state and incidence of new HBV infection. Furthermore, because there are inadequate census data to indicate how the study subjects are representative of an entire village or island population, no effort is made to combine and compare data between the islands where less than 40% of the population was tested; to do so would be an inappropriate pooling of potentially biased samples.

Anti-HBc was measured only in those subjects without either HBsAg or anti-HBs in order to obtain the most complete possible estimates of the extent of HBV infection. Therefore, since anti-HBc was not measured in the entire population, no overall age and sex distribution data for this specific measurement can be reported.

Physical examinations performed on the entire populations of both Maiao (1978 and 1980) and Hiva Oa (1980) yielded no clinical evidence of acute or chronic hepatitis such as jaundice, hepatomegaly, ascites, etc.

Distribution of HBV Infection

The evidence of past or current infection with HBV among the subjects tested on each island is shown in Tables 6-9. On both Hiva Oa and Maiao, greater than 70% of the repsective village populations were sampled.

On Hiva Oa (Table 6), 58% of males, 73% of females or 70% of all subjects were infected. Similarly, HBsAg was found in more females than males (16% vs. 24%) although the sample size was small enough that no clear-cut age distribution could be seen. Anti-HBs was about equally prevalent in both sexes.

The 1978 Maiao results (Table 7) show quite a different distribution of infection. Overall, 95% of males, 83% of females or 88% of all subjects were infected. In both sexes the infection rates were lower in the 1 - 9 year olds and rose to higher levels thereafter. Although HBsAg rates fluctuated between age deciles, in both sexes they rose to a peak in the 30 - 39 year olds and declined rapidly thereafter so that none of the seven persons over 60 years of age had HBsAg. Overall infection rates ranged from 86 - 100% in all but the 1 - 9 year olds, and it can be seen that a low HBsAg prevalence was always counterbalanced by a proportionally higher combined anti-HBs plus anti-HBc prevalence e.g., the 60 year olds with no HBsAg had the highest proportion of antibodies (anti-HBs plus anti-HBc).

The test results from Rapa are found in Table 8. Forty-four percent of the inhabitants were tested and it is impossible to know how these individuals represent the general population. The overall infection rate in this population sample was 96%. Even if none of the remaining 66% of the islanders were infected, the HBsAg prevalence would be 25% at a minimum and the total infection rate would still be greater than 42%. Although theoretically possible, it seems intuitively unlikely that our sample was biased to this extent and therefore it is probable that a major proportion of the Rapa inhabitants were infected by HBV.

Similarly, serum from 40% of Mangareva inhabitants was tested for HBsAg and anti-HBs, although anti-HBc determinations were not made. Results are presented in Table 9. This sample was most heavily distributed in the 10 - 19

and 40 - 49 year old age groups and was probably biased because of this.

Persistence of Infection

A second serum collection was made on the island of Maiao in 1980, nineteen months after the one made in 1978. Although 151 sera were tested in 1978 (Table 7) and 156 in 1980 (Table 10), only 100 individuals were followed and tested at both times. Only 100 persons received follow-up testing because some refused venipuncture on one occasion, were off of the island at one time or the other, or had died between 1978 and 1980.

Table 11 shows the test results of the 100 persons from whom there were paired samples. As was the case with the entire population present in either 1978 or 1980, the infection prevalence was lowest in the 1 - 9 year olds, but nonetheless high at 78%, and remained at a relatively constant high level thereafter.

Table 12 is a summary of the net serologic changes occurring in the group of 100 subjects tested in 1978 and 1980. One 24-year-old male converted from HBsAg to anti-HBs and one 33-year-old female from HBsAg to anti-HBc. Eight persons converted from anti-HBc to anti-HBs and one converted to negative. Of the eight persons negative in 1978 and tested again in 1980, seven converted to anti-HBs (four females and one male less than nine years, one male of eleven years, one of 37 years and one female 54 years of age) and one remained negative (an eight-year old female). This latter group comprised the portion of the population susceptible to becoming infected with HBV and from these results one can calculate the incidence of new HBV infection during the nineteen-month study interval:

$$\frac{7 \text{ (number of susceptibles acquiring infection)}}{100 \text{ (total population)} - 92 \text{ (number already infected)}} = 87.5\%$$

This figure may be converted to an equivalent 55% yearly incidence of HBV infection among those susceptible.

Distribution of HBeAg and Anti-HBe

Table 13 shows the frequency of occurrence of HBeAg and of anti-HBe among HBsAg positive subjects, for each of the islands.

Composition of Subject Pool

Greater than 70% of the inhabitants of the islands of Maiao and Hiva Oa were tested for evidence of HBV infection. An additional 40-41% of inhabitants of two other islands, Rapa and Mangareva, were also tested (Table 5).

Since precise census data were unavailable for any of the islands, it cannot be known for certain whether the subjects tested represent random samples of the respective populations. Because a relatively small proportion of the Rapa and Mangareva inhabitants were tested, the magnitude of cross-sectional sampling errors was uncertain and these individuals and islands were excluded from comparitive statistical analyses. However, they will be dicussed in qualitative terms. On the other hand, a much greater proportion of the Maiao and Hiva Oa inhabitants were tested. Observations made at the time of sample collection on these islands did permit the consideration of possible sampling errors.

In particular, it was likely that the majority of the approximately 30% of untested individuals on Maiao or Hiva Oa were in the 1 - 9 year old age group. No newborn infants and very few children under five years of age were tested, largely due to parental anxiety over the risks of venipuncture. What was felt to be a conservative estimate of the number of young children involved was made by the medical team and suggested to be approximately 50% of the untested subjects of each island. This estimate was derived from comparing the list of persons tested with that of those who had received physical examinations. This latter group comprised essentially the entire village populations.

Looking at the specific numbers involved, on Hiva Oa (Table 6) 13 (11%) of the 120 subjects tested were 1 - 9 years old, so that of the 50 untested subjects, 25 were estimated to also fall into this age group. By adding in this number, it could be calculated that 38 (22%) of the total population of 170 persons were 1 - 9 years old and that those 25 not tested in this age group represented 15% of the total population. The remaining 25 untested persons were randomly distributed among age groups as far as was known and prepresented another 15% of the population.

On Maiao (Table 7), 37 (24%) of the 152 subjects tested were 1 - 9 years old, so that of the 54 untested subjects, 27 were estimated to also fall into this age group. By adding this number, it could be calculated that 64 (31%) of the total population of 206 persons were 1 - 9 years old and that those 27 not tested in this age group represented 13% of the total population. As on Hiva Oa, the remaining 27 were believed to have been randomly distributed among all other age groups and represented another 13% of the population.

Pedigree Analysis

Two family pedigrees were constructed for the population of Maiao (figures 5 - 8). They include 116 (56%) of the 206 inhabitants and are annotated with the results of HBV serologic testing, including those from 65 of the 100 subjects who were tested on two occasions. Cases of unremembered or questionable parentage were eliminated from the pedigrees.

When these pedigrees were viewed in conjunction with the composite population data from Maiao (Tables 7 and 10 - 11), it was seen that from 96 - 100% of the

populations became infected with HBV by the age of ten years. Indeed, of the 18 persons not infected in 1978, 13 were less than 10 years, two 10 - 19 years, one 30 - 39 years, one 40 - 49 years and one 50 - 59 years of age.

It will be recalled that the hypotheses prospectively considered included the possibility that the development of the HBsAg-positive chronic carrier state was genetically determined, that children of HBsAg-positive mothers were more likely to become chronic carriers themselves and that children of HBsAg-positive mothers were more likely to become infected at an early age than those of mothers who were not carriers of HBsAg. Evidence for and against these hypotheses is presented below.

Distribution of Infection Within Families

Looking at all families in both pedigrees, there were two HBsAg-positive mothers. Of their nine tested offspring (ranging in age from 4 - 11 years), four were HBsAg-positive, four anti-HBs positive and one negative by 1980 (family A in figures 5 and 6 and family C in figures 7 and 8). The mother in family A had HBeAg and all four of her progeny had both HBsAg and HBeAg. The mother in family C was e/anti-e negative and all of her children had either anti-HBs (four) or were negative (one). The father of family A had anti-HBs in 1978 and the father of family C was negative in 1978 but had anti-HBs by 1980.

There are two families with HBsAg-positive fathers, B and C in figures 5 and 6. Of the seven children tested (ranging in age from 4 to 11 years), all had developed anti-HBs by 1980 and none had HBsAg. The mothers in both of these families had anti-HBs.

By using the method of chi-square analysis with 2 x 2 contingency tables, it was determined that 1) 1 - 9 year old progeny of HBs-Ag positive mothers were more likely to have HBsAg ($\chi^2 = 4.802$, $n = 30$, $df = 1$, $p < .05$) than were the same aged progeny of other mothers; 2) when progeny of all age groups were taken to-

gether, offspring of HBsAg -positive mothers were still more likely to have HBsAg (X^2 = 4.401, n = 46, df = 1, $p < .05$), but that 3) infection per se was not more likely to occur in 1 - 9 year old progeny of HBsAg-positive mothers (X^2 = .030, n = 31, df = 1, p = ns). The possibility that 1 - 9 year old offspring of an HBsAg-positive father were more likely to acquire HBsAg was also tested and could be rejected (X^2 = 1.974, n = 29, df = 1, p = ns).

Finally, the hypothesis that infection occurred more often in any nuclear family in which any member had HBsAg was rejected (X^2 = 0.065, n = 87, df = 1, p = ns). For the purpose of calculating these probabilities, the assumption was made (53) that those subjects who had anti-HBs in 1978 but were not tested in 1980, in fact still had anti-HBs in 1980. However, no results were inferred when HBsAg data were lacking.

Genetic Mechanisms

Autosomal dominant, x-linked recessive, x-linked dominant and y-linked inheritance of susceptibility to becoming an HBsAg-positive chronic carrier did not appear to operate in this population.

For an autosomal dominant mechanism to be in operation, no infected progeny of a mating between two non-carrier parents should become chronic carriers (i.e., homozygous recessive parents could not produce either heterozygous or homozygous dominant offspring). However, figures 7 and 8 show families A and B to violate this rule since non-carrier parents (161, 124 and 34, 176) had carrier progeny (174, 185 and 36, respectively).

An x-linked dominant mechanism would require that a carrier father have only carrier daughters. Figures 5 and 6 show this not to be the case in families B and C where carrier fathers (55 and 179) had non-carrier daughters (52, 72, 73, 74 and 39, 40 respectively).

In an x-linked recessive model, a carrier mother could have only carrier sons.

Family C in figures 7 and 8 shows a carrier mother (114) with non-carrier sons (107, 110) and thus argues against this mechanism.

Y-linked inheritance would require that a carrier father have only carrier sons. There are numerous instances where this was shown not to be the case. For example, family C in figures 7 and 8 shows a carrier male (179) who was at the same time the son of a non-carrier father and the father of a non-carrier son.

These pedigrees were consistent with the operation of an autosomal recessive mode of inheritance of susceptibility to developing the chronic carrier state. One could postulate either homozygous recessive x heterozygote, heterozygote x heterozygote, or homozygous recessive x homozygous dominant matings in all cases where chronic carrier progeny resulted and thereby account for all of the phenotypes observed in those families. For example, families B and C in figures 7 and 8 could have resulted from homozygous recessive (55 and 179) x heterozygote or homozygous dominant (54 and 42) matings giving all non-carrier children. A heterozygote (161 and 134) x heterozygote (124 and 176) mating in families A and B in figures 7 and 8 could explain the observation of both carrier (174, 185 and 36) and non-carrier (158, 173, 165 and 32, 33) children.

DISCUSSION

As was discussed earlier, an ideal study of HBV epidemiology and the natural course of infection should include testing of all individuals in a given population. Thus, it was planned that this study inlcude the greatest posssible proportion of the individuals living on each island. The practical realities of a study such as this have made it impossible to fully realize such a goal i.e., parents were unwilling to have their youngest children tested, some people were away during our brief, limited study visits, some subjects refused to cooperate, and so forth.

In addition, retrospective testing of previously acquired serum samples, such as those from Rapa and Mangareva, is more likely to yield biased results because of biased collection or sample depletion and alteration secondary to storage and handling problems.

All things considered, the prospectively planned surveys on Maiao and Hiva Oa came reasonably close to complete samplings i.e., 74% and 71% respectively. It was estmiated that 75% of untested subjects on Maiao and Hiva Oa were less than 10 years old. On both islands this was the age group having the lowest infection rate and although it is tempting to assume that those subjects who were tested in fact represented an unbiased sample of their age group, there is no way to be certain. However, if this assumption were correct, then the finding of lower overall infection rates in young children would mean that they comprised the largest pool of uninfected, susceptible individuals, as would be expected for any endemic infection where increasing levels of population immunity would occur with increasing age. If the youngest children had a similar risk of exposure to HBV as other aged persons, one would have to postulate e.g., they had immunologic protection against acquiring infection which older persons did not, perhaps persisting maternally-derived anti-HBs (94). On the other hand, if they were at a

lesser risk of exposure to HBV then that fact alone could account for the decreased prevalence of infection among them. It is interesting that the earliest studies done in the South Pacific found the greatest HBsAg prevalence in young children, (6, 15, 17) while more recent studies using more sensitive assays have had results similar to ours (40-43, 141).

One can certainly imagine that the children who were still physically very dependent on their mothers were at a decreased risk for exposure to HBV-contaminated materials because they were not out playing, had fewer intimate contacts with the population at large, and so forth. If they were exclusively breast fed, and their mothers did not have HBsAg in their milk, then they may have had even fewer opportunities for exposure to HBV.

Of course, the assumption that the 1 - 9 year old subjects who were tested were a random, non-biased sample may be incorrect. For example, one could suppose that some progeny infected by HBsAg-positive mothers were selectively excluded. Since only about 25 children were potentially involved on either Maiao or Hiva Oa, if even one HBsAg-positive sibship of 3 -5 children was excluded it would have made a considerable difference in the prevalence rates.

The second assumption, that the remaining numbers of untested individuals (25 on Hiva Oa and 27 on Maiao) were randomly distributed by age, could also have been incorrect. On Hiva Oa, and to a slightly lesser extent on Maiao, the actual number of subjects in the 20 - 29 year old age group was markedly lower than that in the two younger age deciles. If one corrects for the estimated 25% loss of untested 1 - 9 year old subjects, this difference becomes even more remarkable and more difficult to explain. Was there a precipitous increase in the birth rate within the past 19 years? Was there increased mortality among those 20 - 29 years of age or, was there selective emmigration

or, was the sample biased to exclude selectively some of these persons from testing? The answers to these questions are not known.

Another potential source of error to be considered is that HBV infection itself may have influenced the availability of subjects for testing, presumably because they would have been acutely ill from the disease and physically unable to come for venipuncture, or conversely, more likely to seek medical care, or because there was an HBV infection-associated increased mortality which selectively removed affected individuals. However, not one of the subjects examined on any of the four islands manifested clinical evidence of hepatitis. Furthermore, jaundice ascitis or "liver disease" were problems unheard of by the people of these islands. Clinical hepatitis is extremely rare even in the main hospital in Tahiti (to which the large Tahitian population has access and to which inhabitants of these outlying islands are evacuated in case of severe illness) where authorities cannot recall even a single case of fatal non-alcoholic liver disease, including hepatoma. Of course one cannot be certain whether extensive autopsies were performed in Tahiti and, even though the evacuation option (via radio link) exists on all inhabited islands of French Polynesia, one cannot know how many deaths of unknown cause actually occur. Nevertheless, in the absence of any evidence of physical sequelae of HBV infection, it is hard to imagine in what ways HBV infection per se could have influenced either the inclusion or exclusion of subjects for this study.

Finally, how might selection biases have been reflected in the 100 Maiao subjects tested in both 1978 and 1980 (about 50% [100/206] of the inhabitants)? The distribution of subjects by age group was very similar from year to year except perhaps for the 10 - 19 year old group where there were 28% more subjects (six males and four females) in 1980 (46 vs. 36). However, the HBsAg, anti-HBs and total infection (94% vs 98%) rates were remarkably similar in both years and it is therefore likely that, in both 1978 and in 1980, the persons in the 10 - 19 year old age group were representative of the population at large.

Another difference between 1978 and 1980 was that 93% of males and 83% of females were infected in 1978, compared to 95% and 93% respectively, in 1980. Both a relative (55% vs. 84%) and absolute (12 vs. 16) increase in the number of infected 1 - 9 year olds in 1980 was the main contributing factor. This was consistent with the previously discussed possibility thata biased sample of children in this age group was obtained. Another possible cause was that these differences reflected a real, even if transient, change in the prevalence of acute HBV infection from one year to the other, especially since this was the age group which contained the greatest number of susceptible individuals and seroconverters. One cannot be certain which was the case.

Looking only at those 100 individuals tested in both 1978 and in 1980 (Table 11), the distribution of infected persons was very similar to that of the entire 1978 and 1980 samples. Therefore, the calculation of HBV incidence which was based on this sample is likely to reflect the true incidence among those susceptible persons in the two larger samples. And to the extent that these samples were representative of the entire island population, the calculated 55% to yearly incidence among susceptibles represented the true incidence of HBV infection on Maiao. The numbers of subjects within each age group was felt to be too small to make meaningful calculations of age-specific incidence.

HBsAg prevalence and the chronic carrier state

In the longitudinally followed sample from Maiao (Table 12), 19/20 (95%) of individuals with HBsAg were chronic carriers of the antigen. The person who lost HBsAg was a 33 year old female who had only anti-HBc in 1980. She was either resolving her infection and beginning to make anti-HBs or still had undetectably low HBsAg levels. Furthermore, they represented 19% of subjects tested which was almost identical to the 20% of subjects found to be HBsAg-positive in the

entire 1978 subject pool (Table 7). This strongly suggests that over 90%, perhaps closer to 100%, of HBsAg-positive subjects on Maiao (when tested on a single occasion) were actually chronic carriers of HBsAg and is an assumption similar to that derived from many other studies of populations with high HBV infection rates (e.g., 81, 116, 120, 137).

The HBsAg prevalence on Maiao was 24% among males, 15% among females and 20% overall. In both sexes it rose to a peak in the 30 - 39 year old age group and declined thereafter. This sex distribution pattern is very similar to that noted in other South Pacific studies (6, 15, 17, 41-43, 81). The peak age prevalence occurred in an age group 10 - 20 years older than has been reported in some studies (40-42) in which assay techniques of similar sensitivity to ours were used, although it was similar to that found in another (81). Similarly, infection prevalence (HBsAg plus anti-HBs plus anti-HBc) was higher in males than in females, as has also been found elsewhere (6, 15, 17, 41-43, 81). Again, these differences were not tested by statistical methods due to the unknown effects of possible selection biases.

Mazzur and Jones compared the ratio of surface antigenemia to that of antibody in a Melanesian population (81) and found it to be significantly higher in the population under six years of age (the group which also had the highest HBsAg prevalence). This they took as an indication that children below six years tended to become chronic carriers rather than produce antibody. The HBsAg to anti-HBs ratio on Maiao (in 1978 and on the paired samples) rose from 0.54 in the 1 - 9 year old group to 1.0 in the 30 - 39 year old group and quickly decreased thereafter. This is a similar pattern to that found by Mazzur and Jones although the Maiao peak occurs at a different age. However, they did not measure anti-HBc, the presence of which, at least in the older age groups, may well indicate low level carrier state (53). Adding these values to the numerator of the antigen/antibody ratios

shows that while they still peak in the 30 - 39 year old group, they also remain high thereafter. Thus, for Maiao one cannot conclude on this basis that younger persons tend to become antigen carriers while older persons tend to make antibody when infected. While anti-HBc prevalence on Hiva Oa was merely 2%, the HBsAg/anti-HBs ratio was very similar to that on Maiao with a peak at 30 - 39 years of age.

The situation on Hiva Oa appeared to be different from that on Maiao. Although the combined HBsAg prevalence was 20% as on Maiao, it was greater in females (24%) than males (16%) overall and in almost all age groups. Further, only two (2%) individuals without HBsAg or anti-HBs had anti-HBc, versus 16 (10%) on Maiao. On Hiva Oa, those persons with anti-HBc were in the 1 - 19 year old age groups (suggesting acute infection) whereas they were seen to become increasingly common with age (in both sexes) on Maiao, paralleling gradual decreases in HBsAg prevalence (suggesting they might have been long-time chronic carriers of low-level HBsAg [Table 2]). Anti-HBs was nearly equally common in all ages in both sexes within each island but was slightly less prevalent on Hiva Oa (50%) than on Maiao (58%). This was in keeping with the lower combined overall infection rate of 70% on Hiva Oa as compared with 88% on Maiao.

Sixty-eight percent of the Hiva Oa population had either HBsAg or anti-HBs compared to 78% on Maiao. Two of the remaining 38 individuals (5%) on Hiva Oa compared to 16 of 34 (47%) on Maiao had anti-HBc only. Keeping in mind that anti-HBc prevalence increased with age on Maiao, this observation suggests that there were fewer chronic carriers on Hiva Oa than on Maiao. If this was indeed the case, then the 20% of persons on Hiva Oa with HBsAg could not be indentified with certainty as chronic carriers. The longitudinal data which would resolve this issue are unfortunately lacking for Hiva Oa. However, if these persons

were not chronic carriers then the Hiva Oa population would be unlike many studied elsewhere in the South Pacific and this could account for the unusual age and sex distributions of infection that were found. This could be due to environmental features not readily apparent or to genetic influences.

Rapa and Mangareva

Data from Rapa and Mangareva are difficult to interpret since a relatively small portion of the inhabitants were tested. Nevertheless, Rapa is especially intriguing (Table 8) because the age and sex prevalence of HBsAg, while similar in pattern to that reported eleswhere and found on Maiao, was of a much greater magnitude. If all of the untested Rapa inhabitants had had HBsAg, the HBsAg prevalence would have increased from 58% to 81% and if none had HBsAg, would have decreased to 25%, still a remarkable figure when compared to other studies of South Pacific populations (Table 4). But with a minimum of 25% of the population infective and with a minimum of 42% (168/398) already infected, it is unlikely that very many of the untested individuals had in fact escaped infection. For example, on Maiao and Hiva Oa, where only 20% of the population had HBsAg, at least 65% (134/206) and 49% (84/170) respectively of the entire populations were infected (again, assuming that none of the untested inhabitants were infected, which is the least likely assumption).

Mangareva (Table 9) had the lowest HBsAg prevalence of all four islands (4%) as well as the lowest overall infection rate (36%), although anti-HBc determinations were not included in these calculations. It is doubtful that the tested subjects were randomly distributed since the number in each age group varied considerably and there was a notable paucity of subjects in the 1 - 9 year old group.

HBeAg and Anti-HBe

HBeAg and anti-HBe determinations were made using an agar gel immunodiffusion assay (69), which is capable of picking up only relatively high concentrations of antigen or antibody. It is therefore likely that a significant number of the double-negative results recorded were false negative results. Fifty-six percent of the samples contained neither HBeAg nor anti-HBe (Table 13).

HBeAg and anti-HBe have been measured among HBsAg-positive Micronesians (41) and Polynesians (40). HBeAg prevalence (by RIA) was 11% (no anti-HBe) among Micronesians and 38% (1% anti-HBe) in Polynesians.

Other studies (92, 114) have suggested that HBeAg decreases while anti-HBe increases with age among HBsAg-positive subjects. Again, because of the relative insensitivity of the agar-gel immunodiffusion assay compared with RIA, it is inappropriate to compare our results (Table 13) either in total or by age and sex with those obtained elsewhere in the Pacific.

Because of the suggestion (84) that the presence of HBeAg is associated with increased infectivity, one might have expected to find a greater proportion of HBeAg on those islands where HBsAg prevalence was highest. Comparing Maiao to Hiva Oa, this was certainly the case (45% vs. 4%), but Rapa had 30% HBeAg positive. This is surprising, because even in the least likely case that none of the untested inhabitants of Rapa had HBsAg, Rapa's HBsAg prevalence would still have been greater than Maiao's (25% vs. 20%). Perhaps the amount of HBeAg was different among Rapa samples and below the sensitivity of our assay.

The Genetic Hypothesis

Debates in the literature over the hypothesis that there is a genetic susceptibility to developing the HBsAg-positive chronic carrier state have

centered around whether one can invoke environmental factors and/or non-random contact-associated HBV transmission (7, 10, 82) to account equally well for apparent genetic segregation of carriers. In addition, some authors have purported to have disproved the hypothesis (80, 83, 117, 145) of autosomal recessive inheritance of susceptibility by finding that progeny of carrier fathers and mothers (both supposedly homozygous recessives) developed anti-HBs, not HBsAg, when infected.

The genetic analysis carried out thusfar on Maiao does not resolve these issues. Although the patterns of HBsAg acquisition were found to be consistent with an autosomal recessive mode of inheritance of infection susceptibility, there was no family in the Maiao pedigrees in which both parents could be identified as chronic carriers.

In considering the operation of intrafamilial spread of HBV infection, family C in figures 7 and 8 bears particular comment. The 30 year old mother (114) was a chronic carrier of HBsAg. In 1978, (figure 7) her 37 year old husband (106) was not infected and of the five offspring tested, four were likewise uninfected and one (107, 10 years old) had anti HBs. We see that by 19 months later (figure 8), three of the four uninfected children and the husband had been acutely infected and had made HBsAg. Since these individuals had all presumably been exposed to other persons or environmental factors which transmitted HBV, in the same way as had the entire population, this observation suggests that intrafamilial spread of infection has occurred during the nineteen month observation period. Of the several families in which this mechanism might be expected to have operated e.g., families A, B and C in figures 5 and 6, this was the only one with susceptible

members which was witnessed during our sampling interval. That infection occurred more often in any nuclear family with an HBsAg-positive member than it did in other families was shown not to be statistically significant. It must be remembered, however, that only 56% of the Maiao inhabitants are shown on the pedigree and that only 32% were tested in both 1978 and 1980. With these limitations, it is still hard to avoid the conclusion that intrafamilial spread from HBsAg-positive individuals was occurring on Maiao.

There was evidence that progeny of all ages, and 1 to 9 years old in particular, of mothers with HBsAg were more likely to have HBsAg themselves but that infection per se was not more likely. However, looking back at the pedigrees (figures 5 - 8), one sees that though statistically significant, these conclusions are in part based on only two families which had HBsAg-positive mothers, only one of whom could be identified for certain as a chronic carrier (family C, figures 7 and 8). The 1980 data are missing on mother 192 in family A, figures 5 and 6). Given this limitation, one can say only that the data are consistent with the operation of autosomal recessive inheritance of susceptibility to acquiring HBsAg. The addition of HLA phenotypes to the pedigrees may well resolve the issue of whether a genetic mechanism controls susceptibility to developing the chronic carrier state.

It would be extremely interesting to repeat the Hiva Oa sample collection and to study the natural course of HBV infection over time, as was done on Maiao. It is possible that this would also provide an answer to the question of whether the inhabitants of Hiva Oa are reacting differently to HBV infection that people living on Maiao and elsewhere.

A strong effort could be made to test those individuals on Maiao or Hiva Oa for whom blood serum samples or pedigree data were lacking. If this information were available, it would eliminate most sources of sample bias and could be used

in conjunction with HLA data to carry out a sophisticated, state-of-the-art analysis of the gentic susceptibility hypothesis which would go a long way towards resolving an issue that has been seriously debated for over twenty years.

Regardless of how these genetic issues are finally resolved, it should be recognized that the inhabitants of these islands have the highest HBV infection rates which have ever been reported. This is a truly remarkable finding which begs more thorough investigation. Is there subclinical involvement of the liver? What are the specific environmental and immunologic factors which encourage transmission and maintenance of infection among these people? These are some of the exciting questions which remain to be explored.

Technique	Relative sensitivity for detecting HBsAg	Relative sensitivity for detecting anti-HBs	Ease of performance	Relative cost	Time required for completion (hours)
Immunodiffusion (ID)	1-5	1-10	simple	inexpensive	24-72
Counter-Immunoelectrophoresis (CIE)	5-15	5-10	simple	moderate	2
Complement fixation (CF)	15-20	5-10	moderate	inexpensive	2-24
Immune adherence	20-2 000	50-150	moderate	inexpensive	2
Latex particle agglutination (antibody-coated)	15-100	-	simple	inexpensive	0.1-0.2
Passive haemagglutination and inhibition (PHA, RPHA)	15-20	10 000	moderate	expensive	2
Radioimmunoassay (RIA, SPRIA)	2 000-10 000	10 000 - 1 000 000	complex	expensive	24-120
Immune electron microscopy	1 000-2 000	-	complex	expensive	2-4

Table 1. Techniques for measuring HBsAg and Anti-HBs. Adapted from WHO technical report series (2).

Table 2. Interpretation of Serologic Markers of HBV Infection (from 53)

Pattern	Serologic Reactivity			Interpretation
	HBsAg	Anti-HBc	Anti-HBs	
1	+	−	−	Early (presymptomatic) acute HB
2 a.	+	+	−	a. Acute viral hepatitis or
b.		++		b. Chronic HBsAg carrier state
3	−	+	+	Recovery from HB
4 a.	−	+	−	a. (low titer) long after HBV infection
b.		++		b. (high titer) immediate convalescence from HB of "low level" chronic carrier state
5	−	−	+	Long after HBV infection or immunization with HBsAg

Table 3. Risk Factors in HBV Infections (from 126)

A. *Enhanced Probability of Exposure*

Blood transfusion and other parenteral procedures
Lower socioeconomic class
Sexual promiscuity
Intrafamilial contact
Medical profession

B. *Enhanced Probability of HBsAg Persistence*

Immune defects
Age at primary infection
Sex
Race (?)
Genetic (?)

C. *Both Enhanced Exposure and Persistence*

Birth to carrier-mothers
Maintenance hemodialysis
Institutionalization due to Mongolism

Island	Bougin-ville	Bougain-ville	Malatia, BSIP*	Santa Cruz BSIP*	Santa Cruz BSIP*	New Caledonia	Fiji	Fiji
HBsAg	11%		15%	13%	11%		9%	
Anti-HBc		88%				22%		
Anti-HBs				1%	21%		4%	
HBeAg Anti-HBe								
TOTAL INFECTED		144 (88%)		88/626 (14%)	771 (32%)	61 (22%)	374/2874 (13%)	293 (39%)
RACE	Melanesian	Melanesian	Melanesian	Melanesian	Melanesian	Melanesian	Melanesian	Melanesian + Indian
METHOD	ID+	RIA	ID	IEOP	PHA RPHA	RIA	IEOP	RIA
AUTHOR	Blumberg (17)	Wong (141)	Blumberg (15)	Austin (6)	Mazzur (81)	Wong (141)	Austin (6)	Gust (43)
PEAK AGE	0-19 yrs ♂≅♀ (HBsAg)		0-19 yrs ♂≅♀ (HBsAg)	1-6 yrs ♂≅♀ (HBsAg)	30 yrs ♂≅♀ (HBsAg)	40-50 yrs (Anti-HBc)	1-10 yrs ♂>♀ (HBsAg)	

* British Solomon Islands Protectorate

\+ Please refer to Table 1 for an explanation of testing methods listed in this table.

Table 4. Composite results of HBV survey studies carried out in the South Pacific. These data are generally based on random samples of a small proportion of an island's population.

Island	New Hebrides	Gilbert	Nauru	Ellice	Funafuti	Niue	Niue	
HBsAg	2%	26%	15%	8%	10%	5%		
Anti-HBc								
Anti-HBs		57%	64%	75%	65%	1%		
HBeAg			11%		38%			
Anti-HBe					1%			
TOTAL INFECTED	2/96	186 (83%)	638 (79%)	80 (83%)	574 (74%)	14 (6%)	74%	
RACE	Micronesian	Micronesian	Micronesian	Polynesian	Polynesian	Polynesian	Polynesian	
METHOD	IEOP	RIA	RIA	RIA	RIA	IEOP	RIA	
AUTHOR	Austin (6)	Gust (42)	Gust (41)	Gust (42)	Gust (40)	Austin (6)	Gust (43)	
PEAK AGE		10-19 yrs ♂ > ♀ (HBsAg)	20-29 yrs ♂ > ♀ (HBsAg)	20-29 yrs ♂ > ♀ (HBsAg)	10-29 yrs ♂ > ♀ (HBsAg)	30-39 yrs (HBsAg)		

Table 4 (continued)

Island	Rarotonga	Rarotonga	Wuvalu	Samoa	Samoa	Tahiti	Bora Bora	
HBsAg	5%		13%					
Anti-HBc				62%		42%	54%	
Anti-HBs	1%							
HBeAg Anti-HBe								
TOTAL INFECTED	23 (6%)	(80%)	67/372		75%			
RACE	Polynesian	Polynesian	Polynesian	Polynesian	Polynesian	Polynesian	Polynesian	
METHOD	IEOP	RIA	CIEP	RIA	RIA	RIA	RIA	
AUTHOR	Austin (6)	Gust (43)	Gaxotte (32)	Wong (141)	Gust (43)	Wong (141)	Wong (141)	
PEAK AGE			10-19 yrs ♂< ♀ (HBsAg)	10-15 yrs (Anti-HBc)		0-10 yrs (Anti-HBc)	0-10 yrs (anti-HBc	

Table 4 (Continued)

Island	Total Population	Tested No.	Tested %
Rapa	398	(175)	44
Mangareva*	468	(189)	40
Hiva Oa +	170	(120)	71
Maiao 1978	206	(152)	74
Maiao 1980	198	(156)	79

* Village of Rikitea

\+ Village of Atuona

Table 5. Proportion of the populations tested for evidence of HBV infection.

	MALE					FEMALE					COMBINED				
Age (years)	No.	HBsAg %	Anti-HBs %	Anti-HBc %	Total* %	No.	HBsAg %	Anti-HBs %	Anti-HBc %	Total* %	No.	HBsAg %	Anti-HBs %	Anti-HBc %	Total* %
1 - 9	6	0	(2) 33	(1) 17	(3) 50	7	(3) 43	(3) 43	0	(6) 86	13	(3) 23	(5) 38	(1) 8	(9) 69
10 - 19	27	(4) 15	(16) 59	0	(20) 74	32	(5) 16	(16) 50	(1) 3	(22) 69	59	(9) 15	(32) 54	(1) 2	(42) 71
20 - 29	6	(1) 17	(3) 50	0	(4) 67	5	(2) 40	(2) 40	0	(4) 80	11	(3) 27	(5) 46	0	(8) 73
30 - 39	10	(3) 30	(3) 30	0	(6) 60	9	(3) 33	(4) 44	0	(7) 77	19	(6) 32	(7) 37	0	(13) 69
40 - 49	5	0	(3) 60	0	(3) 60	4	(2) 50	(2) 50	0	100	9	(2) 22	(5) 56	0	(7) 78
50 - 59	2	0	(1) 50		(1) 50	4	0	(2) 50	0	(2) 50	6	0	(3) 50	0	(3) 50
60+	2	(1) 50	(1) 50	0	100	1	0	0	0	0	3	(1) 33	(1) 33	0	(2) 66
TOTAL	58	(9) 16	(29) 50	(1) 2	(39) 58	62	(15) 24	(29) 47	(1) 2	(45) 73	120	(24) 20	(58) 48	(2) 2	(84) 70

* HBsAg plus anti-HBs plus anti-HBc

Table 6. Distribution of infected individuals on the island of Hiva Oa, village of Atuona

Age (Years)	MALE No.	HBsAg	Anti-HBs	Anti-HBc	Total*	FEMALE No.	HBsAg	Anti-HBs	Anti-HBc	Total*	COMBINED No.	HBsAg	Anti-HBs	Anti-HBc	Total*
1-9	15	(5) 33	(7) 47	0	(12) 80	22	(3) 14	(8) 36	(1) 5	(12) 55	37	(8) 22	(15) 41	(1) 3	(24) 65
10-19	19	(1) 5	(14) 74	(3) 16	(18) 95	17	(2) 12	(13) 76	(1) 6	(16) 94	36	(3) 8	(27) 75	(4) 11	(34) 94
20-29	15	(5) 33	(10) 67	0	(15) 100	12	(2) 17	(8) 67	(2) 17	(12) 100	27	(7) 25	(19) 68	(2) 7	(27) 100
30-39	7	(4) 57	(2) 29	0	(6) 86	11	(4) 36	(5) 45	(2) 18	(11) 100	18	(7) 39	(7) 39	(2) 11	(17) 94
40-49	4	(1) 20	(3) 60	(1) 20	(5) 100	10	(1) 10	(8) 80	0	(9) 90	15	(3) 20	(10) 67	(1) 7	(14) 94
50-59	8	(2) 25	(4) 50	(2) 25	(8) 100	4	0	(2) 50	(1) 25	(13) 75	12	(2) 17	(6) 50	(3) 25	(11) 92
60+	5	0	(3) 60	(2) 40	(5) 100	2	0	(1) 50	(1) 50	(2) 100	7	0	(4) 57	(3) 43	(7) 100
TOTAL	74	(18) 24	(43) 58	(8) 11	(69) 93	78	(12) 15	(45) 58	(8) 10	(65) 83	152	(30) 20	(88) 58	(16) 10	(134) 88

* HBsAg plus anti-HBs plus anti-HBc

Table 7. Distribution of infected individuals on the island of Maiao, 1978.

%

Age (Years)	MALE No.	HBsAg	Anti-HBs	Anti-HBc	Total*	FEMALE No.	HBsAg	Anti-HBs	Anti-HBc	Total*	COMBINED No.	HBsAg	Anti-HBs	Anti-HBc	Total*
1 - 9	21	(12) 57	(8) 38	(1) 5	100	7	(7) 100	0	0	100	28	(19) 68	(8) 28	(1) 4	(28) 100
10 - 19	28	(16) 57	(11) 39	(1) 4	100	38	(13) 34	(22) 58	(2) 5	97	66	(29) 44	(33) 50	(3) 5	(65) 99
20 - 29	8	(5) 63	(2) 25	0	88	19	(10) 53	(8) 42	0	95	27	(15) 56	(10) 37	0	(25) 93
30 - 39	8	(7) 88	0	(1) 12	100	8	(8) 100	0	0	100	16	(15) 94	0	(1) 6	(16) 100
40 - 49	9	(6) 67	0	(1) 11	67	8	(4) 50	(1) 12	(3) 38	100	17	(10) 59	(1) 6	(4) 24	(15) 88
50 - 59	4	(4) 100	0	0	100	6	(3) 50	0	(2) 33	83	10	(7) 70	0	(2) 20	(9) 90
60+	4	(2) 50	(1) 25	0	75	7	(4) 57	(1) 14	(2) 29	100	11	(6) 55	(2) 18	(2) 18	(10) 91
TOTAL	82	(52) 63	(22) 27	(4) 5	(78) 95	93	(49) 53	(32) 34	(9) 10	(90) 97	175	(101) 58	(54) 31	(13) 7	(168) 96

* HBsAg plus anti-HBs plus anti-HBc

Table 8. Distribution of infected individuals on the island of Rapa, villages of Ahurei and Area.

MANGAREVA

	MALE					FEMALE					COMBINED				
Age (Years)	No.	HBsAg %	Anti-HBs %	Anti-HBc	Total* %	No.	HBsAg %	Anti-HBs %	Anti-HBc	Total* %	No.	HBsAg %	Anti-HBs %	Anti-HBc	Total* %
1 - 9	1	0	0		0	8	0	0		0	9	0	0		0
10 - 19	32	(2) 6	(9) 28		(11) 34	36	0	(6) 17		(6) 17	68	(2) 3	(15) 22		(17) 25
20 - 29	6	0	(3) 50		(3) 50	10	(1) 10	(5) 50		(6) 60	16	(1) 6	(8) 50		(9) 56
30 - 39	10	0	(1) 10		(1) 10	13	(1) 8	(7) 54		(8) 62	23	(1) 4	(8) 35		(9) 39
40 - 49	15	0	(7) 47		(7) 47	12	(1) 8	(7) 58		(8) 66	27	(1) 4	(14) 52		(15) 56
50 - 59	12	0	(5) 33		(5) 33	13	0	(4) 31		(4) 31	25	0	(9) 32		(9) 32
60+	13	(3) 23	(2) 15		(5) 38	8	0	(4) 50		(4) 50	21	(3) 14	(6) 29		(9) 43
TOTAL	89	(5) 6	(27) 30		(32) 36	100	(3) 3	(33) 33		(36) 36	189	(8) 4	(60) 31		(68) 36

Not Tested

* HBsAg plus anti-HBs

Table 9. Distribution of infected individuals on the island of Mangareva, village of Rikitea

Age (Years)	MALE No.	HBsAg	Anti-HBs	Anti-HBc	TOTAL*	FEMALE No.	HBsAg	Anti-HBs	Anti-HBc	TOTAL*	COMBINED No.	HBsAg	Anti-HBs	Anti-HBc	TOTAL*
1 - 9	20	(2) 10	(14) 70	0	(16) 80	19	(2) 11	(13) 68	(1) 5	(16) 84	39	(4) 10	(26) 67	(1) 3	(31) 80
10 - 19	25	(3) 12	(21) 84	(1) 4	(25) 100	21	(3) 14	(17) 81	0	(20) 95	46	(6) 13	(39) 85	(1) 2	(45) 98
20 - 29	13	(6) 46	(7) 54	0	(13) 100	13	0	(12) 92	0	(12) 92	26	(6) 23	(19) 73	0	(25) 96
30 - 39	10	(2) 20	(7) 70	(1) 10	(10) 100	12	(2) 17	(9) 75	(1) 8	(12) 100	22	(4) 18	(16) 73	(2) 9	(22) 100
40 - 49	6	(2) 33	(4) 67	0	(6) 100	3	0	(3) 100	0	(3) 100	9	(2) 22	(7) 78	0	(9) 100
50 - 59	5	(1) 20	(4) 80	0	(5) 100	4	0	(4) 100	0	(4) 100	9	(1) 11	(8) 89	0	(9) 100
60+	3	0	(3) 100	0	(3) 100	2	(1) 50	0	(1) 33	(2) 67	5	(1) 20	(3) 60	(1) 20	(5) 100
TOTAL	82	(16) 20	(60) 73	(2) 6	(78) 95	74	(8) 11	(58) 78	(3) 4	(69) 93	156	(24) 15	(118) 76	(5) 3	(147) 94

* HBsAg plus anti-HBs plus anti-HBc

Table 10. Distribution of infected individuals on the island of Maiao, 1980.

MAIAO, PAIRED SAMPLES, 1978

Age (years)	MALE No.	MALE HBsAg	MALE Anti-HBs	MALE Anti-HBc	MALE Total*	FEMALE No.	FEMALE HBsAg	FEMALE Anti-HBs	FEMALE Anti-HBc	FEMALE Total*	COMBINED No.	COMBINED HBsAg	COMBINED Anti-HBs	COMBINED Anti-HBc	COMBINED Total*
		%	%	%	%		%	%	%	%		%	%	%	%
1 - 9	10	(3) 30	(6) 60	0	(9) 90	13	(3) 23	(5) 39	(1) 7	(9) 69	23	(6) 26	(11) 48	(1) 4	(18) 78
10 - 19	14	(1) 7	(9) 64	(3) 22	(13) 93	11	(1) 9	(9) 82	(1) 9	(11) 100	25	(2) 8	(18) 72	(4) 16	(24) 96
20 - 29	9	(4) 44	(5) 66	0	(9) 100	10	(2) 20	(6) 60	(2) 80	(10) 100	19	(6) 32	(11) 58	(2) 10	(19) 100
30 - 39	7	(3) 7	(3) 7	0	(6) 86	9	(2) 22	(5) 56	(2) 22	(9) 100	16	(5) 31	(8) 50	(2) 13	(15) 94
40 - 49	5	(1) 20	(3) 60	(1) 20	(5) 100	4	0	(4) 100	0	(4) 100	9	(1) 11	(7) 78	(1) 11	(9) 100
50 - 59	3	0	(3) 100	0	(3) 100	2	0	0	(1) 50	(1) 50	5	0	(3) 60	(1) 20	(4) 80
60+	2	0	(2) 100	0	(2) 100	1	0	0	(1) 100	(1) 100	3	0	(2) 67	(1) 33	(3) 100
TOTAL	50	(12) 24	(31) 62	(4) 8	(47) 94	50	(8) 16	(29) 58	(8) 16	(45) 90	100	(20) 20	(60) 60	(12) 12	(92) 92

* HBsAg plus anti-HBs plus anti-HBc

Table 11. Distribution of 1978 Maiao subjects from whom paired samples were tested in 1980.

	1978 No.	1980 No.	Net Change	Explanation
HBsAg	20	19	-1	converted to anti-HBC
Anti-HBs	60	77	+17	9 converted from anti-HBc 8 converted from negative
Anti-HBc	12	2	-10	8 converted to anti-HBs 1 converted to negative
Negative	8	2	-6	converted to anti-HBs
TOTAL	100	100	0	

Table 12. Distribution of seroconversions among the Maiao subjects tested in both 1978 and 1980.

Island	No. HBsAg Positive	Tested		HBeAg		Anti-HBe		Neither	
		No.	%	No.	%	No.	%	No.	%
Rapa	101	(76)	75	(23)	30	(13)	17	(40)	53
Mangareva	8	(8)	100	(3)	37.5	(2)	25	(3)	37.5
Hiva Oa	24	(24)	100	(1)	4	(4)	17	(19)	79
Maiao 1978	30	(29)	97	(13)	45	(1)	3	(15)	52
TOTALS	163	(137)	84	(40)	29	(20)	15	(77)	56

Table 13. Frequency of HBeAg and anti-HBe among all subjects tested who were HBsAg positive.

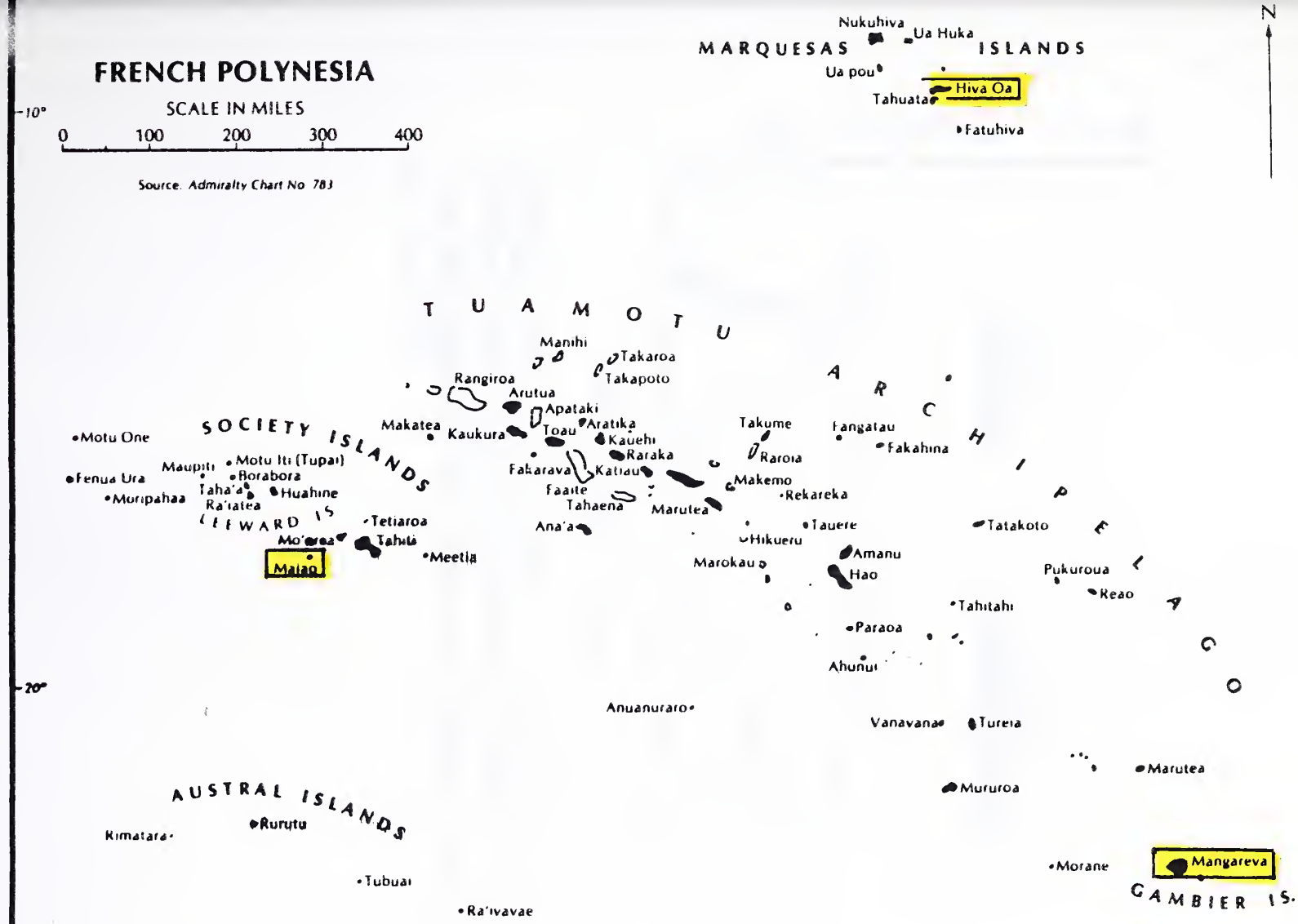

Figure 1. Map of French Polynesia.
From: Tahiti Nui, by Colin Newberry,
University Press of Hawaii, Honolulu,
1980.

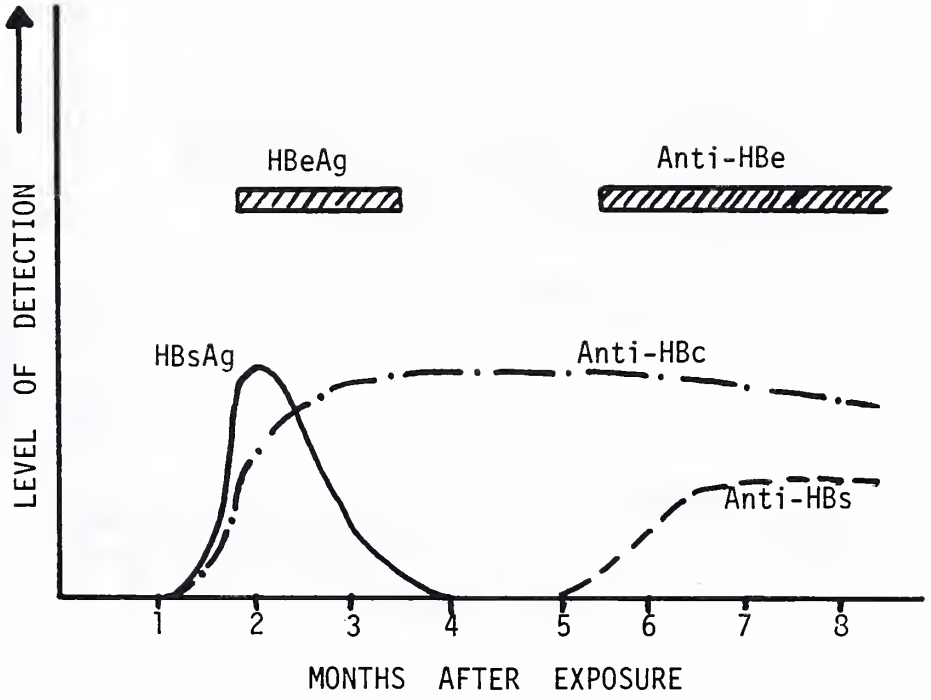

Figure 2. The serologic responses of an individual with a transient HBsAg response after exposure to HBs-Ag positive serum. From: Maxcy-Robinson Public Health and Preventative Medicine, by JM Last (67)

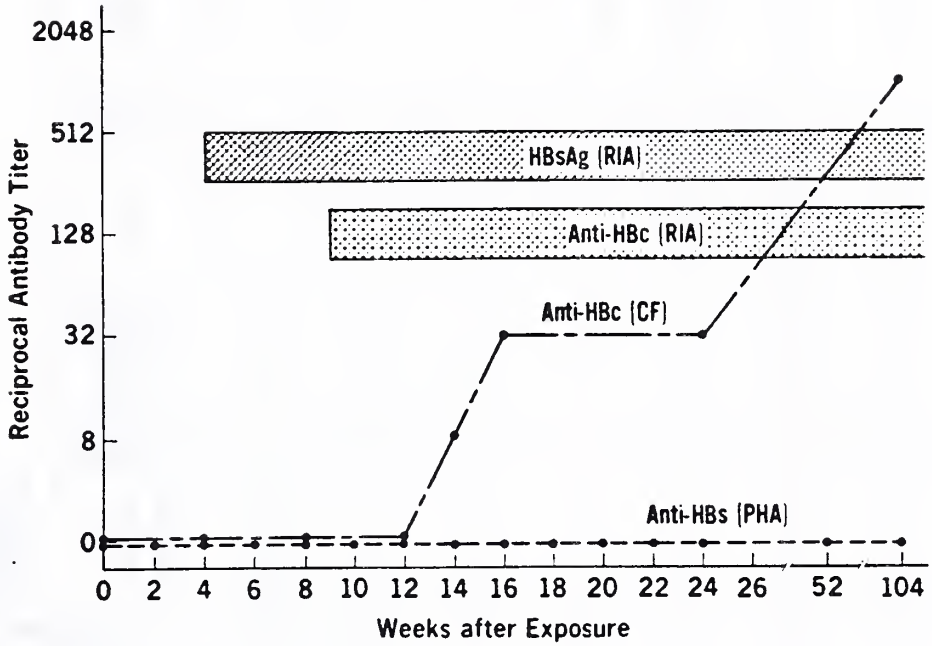

Figure 3. Serologic responses of an individual who developed the HBs-Ag positive chronic carrier state. From Hoofnagle et al, 1978 (53).

INSTITUT DE RECHERCHES MÉDICALES LOUIS MALARDÉ

Numéro de famille ☐☐☐☐

Personne intérrogée ☐☐☐

Année des dernières nouvelles
000 . jamais
999 : relations permanentes

Jumeau : 1 : N, 2 : 0

D. numéro	Numéro de ligne	Nom, prénoms	Degré de parenté: Personne interrogee	Conjoint	Parent/Parent de fr.-sr	Oncle/tante	Grands-parents	Frère / sœur	Demi-fr. / demi-sr.	Enfant	Enfant du conjoint	Conjoint de l'enfant	Petit enfant	Neveu, nièce	Parent du conjoint	Fr./sr du conjoint	Cousin, cousine	Conjoint de fr./sr	Sexe	Jumeau	L'ordre de naissance	Date de naissance Mois / Année	Toujours en vie	Année du décès	Année des dernières nouvelles	Nom du père	Père de sang	Nom de la mère	Mère de sang	Nom du conjoint	Enfants ♂ reconnus	Enfants ♀ reconnus	Enfants non reconnus
			01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	M F		__ __	__ __ / __ __ __	N 0 ?	__ __ __	__ __ __	☐☐☐☐	N 0 ?	☐☐☐☐	N 0 ?	☐☐☐☐			__
			01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	M F		__ __	__ __ / __ __ __	N 0 ?	__ __ __	__ __ __	☐☐☐☐	N 0 ?	☐☐☐☐	N 0 ?	☐☐☐☐			__
			01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	M F		__ __	__ __ / __ __ __	N 0 ?	__ __ __	__ __ __	☐☐☐☐	N 0 ?	☐☐☐☐	N 0 ?	☐☐☐☐			__
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			01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	M F		__ __	__ __ / __ __ __	N 0 ?	__ __ __	__ __ __	☐☐☐☐	N 0 ?	☐☐☐☐	N 0 ?	☐☐☐☐			__
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10	11	12-44	45-46																47	49	49-50	51-55	56	57-59	60-62	63-66	67	68-71	72	73-76	77	78	79

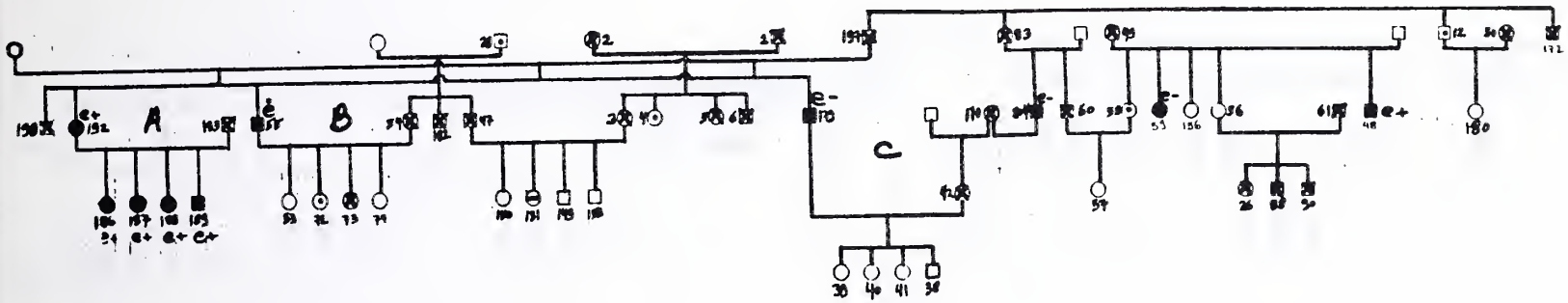

Figure 5. Pedigree for the island of Maiao, 1978

- HBsAg-positive
- Anti-HBs-positive
- Anti-HBc-positive
- Negative
- Not Tested

e+ - HBeAg positive
e - Anti-HBe positive
e- - Negative for e/anti-e

□ = male ○ = female

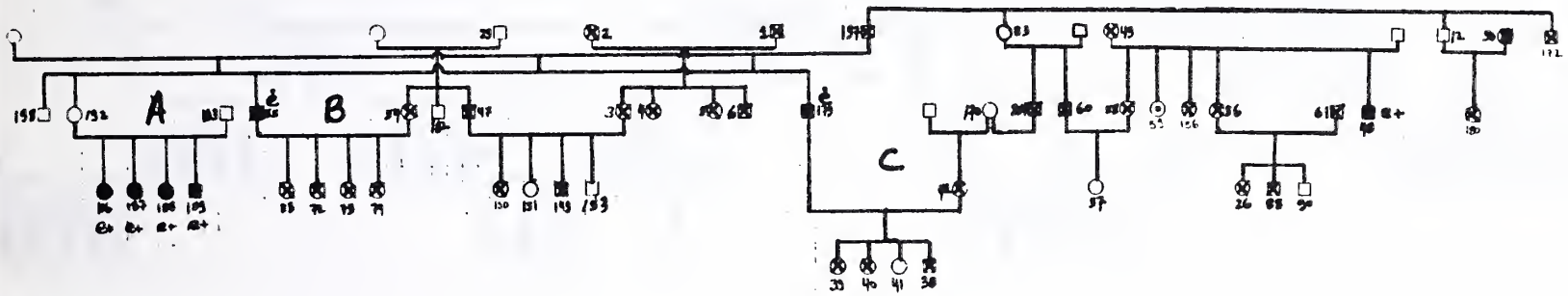

Figure 6. Pedigree for the island of Maiao, 1980
(see figure 5 for key to abbreviations)

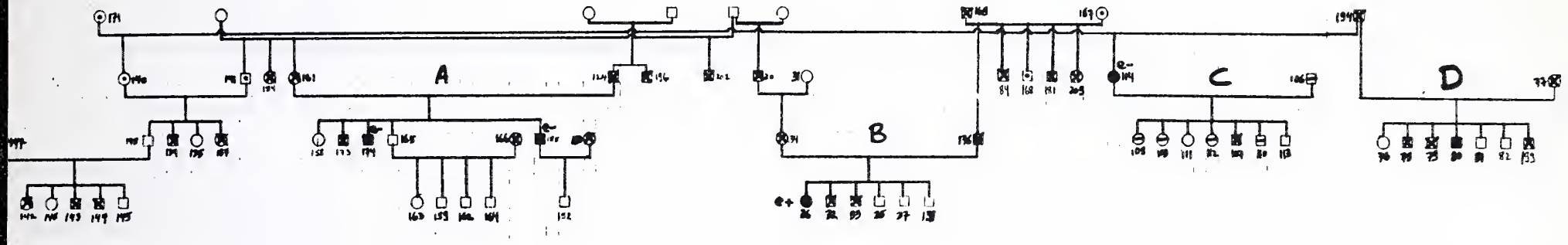

Figure 7. Pedigree for the island of Maiao, 1978
(see figure 5 for key to abbreviations)

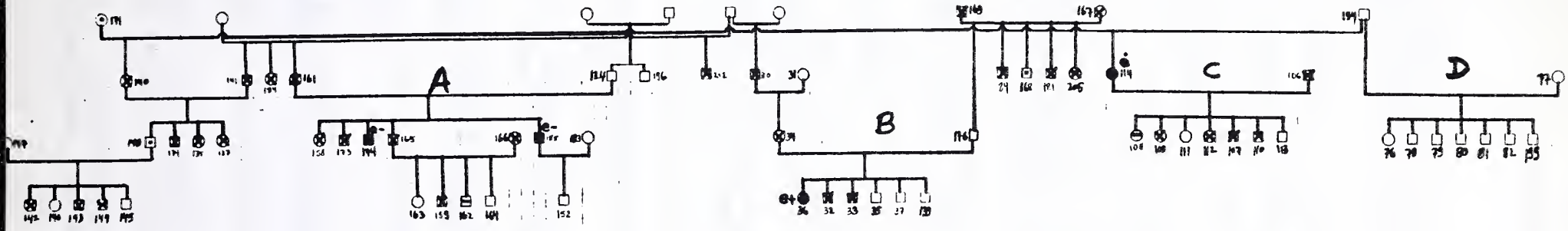

Figure 8. Pedigree for the island of Maiao, 1980
(see figure 5 for key to abbreviations)

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